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CARCINOIDS (ARGENTAFFIN-CELL TUMORS) AND NERVE HYPERPLASIA OF THE APPENDICULAR MUCOSA*

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In 1924, under title of "Neurogenic Appendicitis and Carcinoids," I reported the result of studies commenced in 1913 of more than 400 appendices, some healthy, others with pathological history. I showed that the latter contain neuromata, which perhaps explain the pain of chronic appendicitis. These neuromata arise from the nerves of the periglandular plexus; they form after the migration, into the nerves, of cells containing chromaffin and argentaffin granules which are similar to the granules of the chromo-argentaffin cells of the normal epithelium. I showed further that carcinoids arise from certain intranervous argentaffin cells.

Various considerations lead me to believe that the periglandular plexus from which these neuromata arise is not of sympathetic origin but that it depends genetically on certain entodermic cells and that it represents a *placode*, a *neurentoderm*. This opinion has been rejected as heretical, although the observations on which it is based have been confirmed by many investigators; but no one has been willing to review the question as a whole and with the methods which I recommended.

I return to the subject now because I have controlled my former conclusions by the study of 800 more appendices and because, far from contradicting these conclusions, this study has confirmed them and has enabled me to reply to various objections. I shall report the researches in chronological order; in this way the reader will best understand how and why they led to conclusions which at

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first sight seem revolutionary. My warmest thanks are due to my friend, Dr. George F. Laidlaw, who assumed the task of translating this paper for the Journal.

I. Carcinoids

I shall not pause here to review the literature that has been inspired by these curious tumors.* Often multiple, they have been found along the entire length of the alimentary canal from the stomach to the rectum; but their favorite situation seems to be the appendix. Although not strictly identical in every detail, nevertheless, their structure has so many features in common that they may be grouped under the same title, no matter what their situation in the alimentary canal. Thus it is very probable that their point of departure and their causes are the same everywhere. In this paper, I shall consider only the appendicular carcinoids, basing their description on 50 specimens which I have studied personally.

Appendicular carcinoids may form in the body of the appendix where, in growing, they may cause a stenosis of the lumen with retention, followed by acute inflammation. More often, in 46 of the 50 specimens in my collection, they are found at the distal end. If the carcinoid is small, the tip of the appendix is not deformed; if large, it is swollen like a pendulum. This swelling is often the only sign of a possible appendicular tumor but sometimes the presence of tumor is indicated by an opaque, yellowish infiltration of the peritoneal connective tissue and fatty lobules that cover the tip of the appendix.

Section of the appendix shows that the lumen of the tip has disappeared and that it has been replaced by compact tissue, more or less fibrous, hard, yellowish or brownish. To the naked eye the muscular layers seem to be almost intact and always recognizable, even when distended by the tumor and even when the tumor has infiltrated them before involving the peritoneum.

Where the tumor is situated there is no longer an appendicular lumen. The lumen may appear immediately above the tumor; here we may believe that the tumor has caused the obliteration. Sometimes the tumor is separated from the lumen by a variable space where the naked eye perceives nothing but fibrous tissue and

* For the literature, consult the excellent monograph of Wiley D. Forbus, *Bull. Johns Hopkins Hosp.*, 1925, xxxvii, 130.

where subsequent microscopic examination reveals no carcinoid cells. This observation is of the first importance for it shows that carcinoids may form in a long stenosed region of the appendix, long deprived of its mucosa and consequently of an epithelial lining.

Histology of Carcinoids: From their structure carcinoids were at first thought to be carcinomata; in fact, they are constituted by columns and masses of epithelia which infiltrate. Their usual benignity and their small size earned for them the name little carcinomata (Lubarsch), then carcinoid (Oberndorfer), under which name they are commonly described. We shall take as a type the carcinoids which form at the tip of the appendix.

When the carcinoids are small, the epithelial columns are situated solely in the axial connective tissue of the appendix; in other specimens, while situated in the axial connective tissue, they invade also the interstices of the circular and longitudinal muscle coats and the nerves of Auerbach's plexus without destroying either the contractile fibers or the nerves. In still other more advanced specimens, they occupy the axis of the appendix, the interstices of the muscle and invade the peritoneal fat. It is obvious that their point of departure is always the axis of the appendix * and that they infiltrate the muscularis and the serosa later. Their development is centrifugal.

Carcinoids consist of epithelial cells grouped in columns of varying breadth and a stroma. We shall study these elements successively in preparations made by the usual methods. The cells are small and of various forms which can be arranged in three groups: round cells, which may be polygonal from reciprocal pressure; palisade cells; and columnar or, better, prismatic cells. Mitoses are rare but not amitotic figures.

Round or polygonal cells are much the most common, forming columns of varying breadth. Those next to the connective tissue are cuboid. The nuclei are central, round and turgid, the network finely reticulated and dotted with minute chromatic granules. In well fixed preparations, the protoplasm is clearly bounded by a

* In a paper which appeared in 1914 and which has only recently come to my attention, Ehrlich supposes that carcinoids arise from Auerbach's plexus. On this supposition he bases his statement that these tumors are immature sympathetic neurocytomata. We shall see why this interesting view is inadmissible. For the moment, we note that it rests on insufficient evidence, for Ehrlich saw only advanced carcinoids which had already infiltrated the muscularis and the plexus myentericus.

very delicate membrane. In some cells the protoplasm is homogeneous and frankly acidophil, in others it is abundant and dotted with acidophil granules of extreme delicacy and great regularity. Most of the cells contain tiny vacuoles, sometimes few, sometimes very many and of uniform size like spongiocytes of the adrenal cortex or xanthoma cells (Fig. 29).

Palisade cells (Figs. 28, 30) are less constant. Usually they collaborate with the round cells in forming the columns, ranging themselves along the connective tissue as on a basement membrane. The nuclei present the same texture as those of the round cells; they are turgid, without puckering, and oval in shape. The nucleus is at some distance from the foot of the cell and all this infranuclear region of the cell is filled with acidophil granules, among which may sometimes be distinguished a tiny diplosome surrounded by a nongranular area. This basal and granular region of the cell has no vacuoles but the vacuoles appear about the nucleus and are often very abundant in the supranuclear region.

Sometimes the palisade cells undergo a sort of stratification; some are prismatic, others remain attached to the connective tissue by a long, slender, granular foot while the nuclear and swollen part of the cell insinuates itself deep among the polygonal epithelia. The cell has the form of a tennis racket, only the broad part containing the vacuoles (Fig. 30). Other cells, attached to the connective tissue by a slender foot, are pointed at the other end also, becoming fusiform.

The columns may consist of palisade cells only, curving inward and resembling remarkably the columns of the glomerular layer of the adrenal cortex of certain animals, the horse, for example.

The columnar or prismatic cells are always grouped around a small round cavity or vesicle, forming a rosette (Figs. 28, 31). The cavity may contain a tiny albuminous droplet, more or less colorable, or a homogeneous droplet of colloid aspect. The apical pole of the cell bounds the cavity; this pole is narrow, always bordered by Kittleisten, and consists of a membrane which may be very thin or may be thickened as a striated cuticle. The basal pole rests on the connective tissue or on the polygonal cells. Laterally, the cells are separated by delicate smooth membranes, well outlined. The oval nucleus, similar to those of the palisade cells, occupies the middle of the cell.

The apical pole of each cell is clear, without granules; a tiny diplosome may be detected near the tip of the cell. In some specimens all this supranuclear region is vacuolated. The basal pole is always loaded with granules and is without vacuoles.

These cell forms are connected by all possible intermediary forms and they are often associated in the same column. It is obvious that they do not represent distinct species but rather forms of differentiation of one and the same strain, characterized by nuclei of special structure and by protoplasm which is fundamentally clear and only slightly colorable, in which are often found very tiny acidophil granules and vacuoles, at times numerous but always small and irregular. Moreover, these inclusions which are diffused through the polygonal cells are clearly polarized in the palisade cells. The granules accumulate especially in the basal or connective tissue pole, the vacuoles in the opposite pole. The columnar cells are still more clearly oriented, for the basal pole alone contains granules while the apical pole never has them. Thus the palisade cells and the columnar cells have a granular basal or connective tissue pole and a homogeneous vacuolated apical pole. This orientation is particularly striking in certain specimens where massive columns are penetrated by fine blood capillaries; each of the capillaries is surrounded by a radiating rosette formed by the granular bases of the cells implanted on it.

Beyond the vascular areas just described, the stroma of carcinoids is fibrous or fibro-hyalin, very rich in delicate elastic fibers. There are found also arterioles and venules with muscular walls which scarcely exist in the stroma of carcinomata.

Finally, in that portion of the tumor which develops centrally, inside of the muscular wall of the appendix, smooth muscle fibers are often found among the connective tissue and elastic fibers of the stroma. These may appear in such quantity that they alone form almost all of the stroma, to such a degree that the tumor may be called an adenomyoma or myocarcinoid. Highly important is the fact that this myomatosis does not arise from hyperplasia of the longitudinal or circular layers of the muscle coat; there is always to be found a purely fibrous layer derived from the submucosa which isolates it from the muscularis.

The myomatosis has but three possible sources, hyperplasia of the muscle coat of the arteries or veins, production of muscle fibers

by the connective tissue cells of the stroma, or hyperplasia of the muscularis mucosae. I am inclined to accept this last hypothesis; for the myomatosis is found only in the axis of the appendix, the situation of the fibers of the muscularis mucosae, and not in the longitudinal or circular layers of the muscle coat or in the peritoneal fatty connective tissue when this is invaded by the carcinoid epithelia. In short, the carcinoids seem to have an elective action on the proliferation of the muscularis mucosae.

To sum up their histology, carcinoids resemble ordinary carcinomata of the intestine in the cylindrical form of certain cells; they differ by vacuolation and by the fine granulation of their protoplasm, the granules often accumulating in the vascular pole of the cell.

The Nature of Carcinoids: In 1912, Saltykow, struck by this vascular arrangement of the granules, by their acidophilia and by the endocrine structure of carcinoids, believed them to be tumors arising from aberrant islands of Langerhans. This explanation should never even have been considered for as early as 1907 Oberndorfer had demonstrated that the granules of carcinoids are chromaffin and that their vacuoles are filled with doubly refracting lipoids. Now, the fine granules in the cells of the islands of Langerhans are not chromaffin and these cells never contain doubly refracting fats.

In 1910, Huebschmann advanced the hypothesis that the carcinoids arise from the cells of Paneth or, more probably, from the granular cells discovered in the intestinal epithelium by Nicolas, rediscovered by Kulchitzky, studied later by Schmidt (yellow cells), then by Ciaccio (enterochromaffin cells); but he went no further.

Specific Vacuoles and Granules: On beginning my own studies of carcinoids I found the vacuoles and the granules. In frozen sections the vacuole is seen to be filled by a droplet which stains bright red with scarlet red and with Sudan. The droplets themselves are not doubly refractive but contain crystals which look bright with crossed Nicol prisms. It is probable then that the droplets consist of a mixture of neutral fats and cholesterin esters. Ciaccio's method shows that the walls of the vacuoles contain a very small quantity of lecithin.

From this point of view the fatty inclusions of carcinoids recall those of the cells of the adrenal cortex. On the other hand, the granules are chromaffin and stain black with iron hematoxylin like those of the adrenal medulla. Thus, by the contents of their vacuoles

and by the chromaffin and siderophil reactions of their granules, the carcinoid cells resemble both the cells of the adrenal cortex and those of the medulla and present the features of both in one and the same cell. Now, it is acknowledged that from an embryological, an anatomical, and a functional point of view the adrenal medulla and cortex, the one of sympathetic origin, the other of coelomic origin, are absolutely distinct. Moreover, neither the adrenal nor its tumors ever present prismatic cells grouped in rosettes around a cavity filled with colloid. Therefore, the carcinoids cannot arise from adrenal inclusions.

Next resorting to a new technique which I had already used in the study of melanin, sections of material fixed in the picro-formol of Bouin were immersed in Fontana's ammoniacal silver nitrate. I found that the granules of the carcinoid cells stained dark brown and consequently possessed argentaffin and silver-reducing properties. Sections of adrenal medulla treated in the same way remained colorless, which observation completed the proof that there is no possible identity between carcinoids and the adrenal paraganglion. But this technique applied to sections of the intestine brought out with great clearness the granules of the cells of Kulchitzky.

Thus it became almost certain that the carcinoids arose from a pure proliferation of the enterochromaffin, argentaffin and silver-reducing cells. Since the structure of these tumors resembles that of certain endocrine glands (Saltykow), I called them endocrine tumors and suggested that the chromo-argentaffin cells of the intestine constitute a diffuse endocrine gland.

Since my studies of 1914, a number of researches have been made on carcinoids and on the chromo-argentaffin cells of the intestine. Concerning carcinoids, the opinions may be divided into two groups according to whether or not the writers were familiar with my publications. Thus Abrikossow, following Aschoff, believes them to be nevi of the intestinal mucosa. Krompecher holds them to be basal epitheliomata. Schober groups them among the "progonoblastomata" of Mathias. Engel and Lauche attribute them to embryonic inclusions. Ehrlich makes of them "immature neurocytomata" of the sympathetic.

Those writers who have been willing to adopt my silver technique or any method in which formol fixation is followed by immersion in an ammoniacal silver salt (Delbet and Herrenschmidt, Danisch,

Hasegawa, J. F. Martin, W. D. Forbus, Sprafke), have agreed with me that these tumors arise from the chromo-argentaffin cells of the intestinal epithelium.

As for their endocrine nature and the endocrine function of the cells of Nicolas-Kulchitzky, most writers have refused to follow me. In repeating my experiments on the cells of Kulchitzky, histologists and pathologists have confirmed most of the observations made by my forerunners and by myself; but they have interpreted them in a different manner. Let us study the cells of Kulchitzky as they may be observed in the lining of the alimentary canal.

II. The Cells of Nicolas-Kulchitzky

Yellow cells (Schmidt); *enterochromaffin cells (Ciaccio)*; *argentaffin or silver-reducing cells (Masson)*; *chromo-argentaffin cells (Cordier)*: The cell of Nicolas-Kulchitzky is characterized chiefly by its granules and secondarily by a special form and structure; for, under certain circumstances and perhaps in certain functional states, the granules may be absent. Post-mortem changes destroy the granules more or less completely, hence the necessity of using strictly fresh material.

The fixative should always contain formol but no alcohol (Masson, old but unpublished studies; Hamperl, Cordier). Formol itself fixes the granules well but fixes the tissues very badly.* The best fixatives are Bouin's picro-aceto-formol and the bichromate-formol mixture. Bichromate gives to the granules a yellowish brown tint; after picro-formol or formol alone, the granules are invisible because of their special refraction but they stain intensely with ammoniacal silver nitrate, with iron hematoxylin and with acid dyes, such as eosin, acid fuchsin and ponceau de xylidine, as will be described more fully in the section on Technique.

The alimentary canal of all vertebrates contains granular cells similar to the cells of Nicolas-Kulchitzky. In mammals, they are found from the cardia to the anus (Masson, unpublished studies; Hamperl, Cordier), the form varying somewhat with the species and the region. In the gastric glands and in Brunner's glands, these cells insert slender prolongations between the bases of the gland cells; in the human intestine, and consequently in the appendix, their characteristics are as follows (Fig. 1).

* I am perhaps alone in this opinion but the greater my experience the more I am convinced of its truth.

They are scattered singly among the cylindrical cells of the intestinal epithelium, from five to ten in each gland of Lieberkühn, where they are most numerous. They are less frequent in the upper part of the tubule but are found as far as the tip of the villi, where they may be seen desquamated like the other cells of the intestinal mucosa. It is probable that those deep in the tubule are fixed and that only those placed more superficially migrate with their neighbor cells and disappear like them.

In form, the cells of Nicolas-Kulchitzky are almost always conical, with a broad base in contact with the glandular basement membrane, and with a narrow apex bounded by a delicate membrane which is surrounded by the apical membranes of adjacent cells (cells of Paneth, cylindrical cells, caliciform cells). Some of them border the gland lumen, the bulk of the cell being buried among the other epithelia.

The nucleus is round or, more often, oval, turgid and regular in outline except that in some cells the basal pole is hollowed like a cup. The nucleus is never in contact with the basement membrane but rather in the middle of the cell. The nucleus is clear; it contains one or two karyosomes and a network finely dotted with chromatin.

The protoplasm is clear and homogeneous, staining less deeply than the other epithelial cells. There is a Golgi apparatus in the supranuclear region (Cordier) and another in the basal region (Kull). The basal region almost always contains tiny granules which are acidophil, chromaffin, stain with Heidenhain's iron hematoxylin, reduce silver and turn greenish with basic blues. The granules vary in quantity; sometimes they are all beneath the nucleus, sometimes beneath and at each side of the nucleus. Rarely a few are found above the nucleus but never outside of the cell in the intestinal lumen (Cordier). They seem to disappear at certain functional stages of the cell (Cordier).

The granules present a different appearance according to the technique employed. If blocks of tissue fixed in bichromate are treated with silver before embedding (see Technique), the granules are comparatively large, all of the same size and equally black. In paraffin sections treated with silver the granules vary greatly; some of them reduce silver strongly or stain intensely with iron hematoxylin and among these granules some are large and others barely visible; other granules stain neither with silver nor with hematoxy-

lin but with acid dyes. This variable staining may be seen in one and the same cell and also in different cells of the same gland of Lieberkühn, some cells containing more black granules, some more acidophil granules.

In my opinion, these differences in size and staining in tissue treated with silver before or after embedding, depend on the dissolving out by the toluol of some substance which at a certain stage forms part of the granule. Of the nature of this substance, many guesses might be made. Doubtless these variations correspond to different stages of evolution of the granules. From this standpoint it is well to note them but more than this it is impossible to say.

Besides the granules, the basal protoplasm may contain one or two vacuoles, the contents of which stain with Sudan (Danisch).

Thus, in every feature, the cells of Nicolas-Kulchitzky are identical with the cylindrical and argentaffin cells which group themselves as vesicles in the carcinoids. The palisade, fusiform and spongy cells of these tumors are not found in the normal epithelium; doubtless they represent a morphological deviation which depends on their peculiar situation in the interstices of the connective tissue.

Origin of the Normal Argentaffin Cells: My embryological studies, confirmed by those of Parat, have shown me that the argentaffin cells appear in the intestinal epithelium about the fourth month of fetal life in man; they seem to spring directly from the cells of the entoderm. Kull, however, studying them in the chick embryo by a mitochondrial method, not by ammoniacal silver, derives them from mesenchyme cells that have invaded the epithelium. This opinion cannot be maintained; the very figures published by Kull forbid it. There is not the slightest possible resemblance between the mitochondria of the connective tissue cells of the mucosa and the chromaffin granules of the chromo-argentaffin cells, although these latter also stain with Altmann's acid fuchsin. Their dimensions are very different.

Influenced by my own work (see Argentaffin-cell Neuroma, page 192), Danisch seeks to show that the chromo-argentaffin cells arise from the solar plexus and migrate to the intestinal epithelium in the fourth month of fetal life. His figures are not very convincing and, according to his own statement, represent macerated tissue. Moreover, Danisch uses Agdhur's silver technique which indeed colors the argentaffin cells but not specifically and colors also certain

chromaffin cells of the sympathetic paraganglia. His conclusions should be rejected.

There are two pathological observations which tend to show that the chromo-argentaffin cells are really of entodermic origin:

1. In chronic gastritis, they are found in abundance in those glands of intestinal type which form in regeneration of the mucosa.

2. They are sometimes found (Hamperl, Martin and Masson) in cancers of the intestinal tract and in their metastases, mixed with ordinary cylindrical cells, and may be traced easily to their origin in the common type of cylindrical cell.

Function of the Argentaffin Cells: The granules of the cells of Nicolas-Kulchitzky have inspired many physiological hypotheses. Ciaccio holds that, because of their chromaffinity, the cells produce adrenalin which is poured into the intestinal canal. However, the specific reaction to ammoniacal silver indicates that the substance secreted differs from adrenalin. The chromaffinity and the silver reduction are proofs of reducing power; more than this cannot be said at present.

The situation of the granules at the base of the cell indicates an endotropic polarization of the cell; for this reason I have advanced the idea that they have an endocrine function. In their normal state this function is doubtless not exclusive. Kull states that the cells have two Golgi apparatuses, one apical, the other basal. According to Cowdry, the situation of the Golgi apparatus is connected with the pole of discharge of the cell. Moreover, in the carcinoids, the presence of cavities of secretion bordered by cylindrical cells and filled with an albuminous or colloid liquid, demonstrates that these cells secrete something from their apical pole; in a word, they are exocrine.

However, this does not exclude a concomitant endocrine function. Liver cells normally exhibit two polarities, external and internal. The pancreatic cell is capable of exhibiting them successively and, to return to the carcinoids, it is obvious that if the palisade and the polygonal cells of the larger masses secrete anything, either they retain it in their interior or eliminate it into the vessels. If then the cylindrical argentaffin cells of the normal intestine and of the carcinoids secrete in both directions, the cells of the same lineage which do not border the lumen of a tubule have certainly lost their exocrine function and can be only endocrine. This endocrine function is,

perhaps, not without some connection with the proliferation of the muscle fibers which is so often observed in the stroma of carcinoids.

Cordier has expressed doubt of the endocrine function of the cells of Kulchitzky; in asserting their exocrine function, he relies on the disappearance of the granules after injection of pilocarpin. I do not deny this disappearance but it proves nothing against my thesis; for Cordier has been unable to show in which direction, intestinal cavity or interstitial tissue of the mucosa, the product which results from the destruction of the argentaffin granules is excreted.

In sum, our knowledge of the normal chromo-argentaffin cells shows their identity with the cells which constitute carcinoids; but their physiological rôle remains obscure and their endocrine function in particular remains to be proved.

III. Argentaffin-cell neuroma

(*A*) argentaffin-cell neuroma in obliterated appendices: If carcinoids are really endocrine tumors composed exclusively of enterochromaffin and silver-reducing cells, there is reason to believe that the normal enterochromaffin cells, when situated in the intestinal epithelium, possess similar properties and functions, and that these are endocrine. How shall we verify this?

There comes to mind Laguesse's famous experiment in which he demonstrated the endocrine function of the islands of Langerhans by ligating the external pancreatic ducts. The exocrine acini atrophied rapidly; the islands persisted. At one stroke, he demonstrated their purely endocrine function and their rôle in the metabolism of glucose by the elaboration of a substance which has since received the name insulin. Unfortunately, an experiment of this kind cannot be carried out on the intestine, especially the appendix, ligature of which would be followed immediately by subacute inflammatory symptoms. At this point, I thought of utilizing the experiments performed by nature and examined appendices in which the lumen had been obliterated by cicatricial stenoses, or such as were so considered by most writers, in the hope of finding argentaffin cells in that axial connective tissue which replaces the former lumen and the mucosa.

If the accidents of cicatrization had incarcerated fragments of the glands of Lieberkühn and isolated them from the intestinal cavity,

the argentaffin cells, if really endocrine, might survive the others and persist in the connective tissue just as the islands of Langerhans persist after disappearance of the acini.

My first studies in this direction did not confirm this anticipation but they enabled me to draw three unexpected conclusions:

1. In the axial connective tissue of obliterated appendices there is always to be found a discontinuous, more or less prominent, muscular sheath formed by the persistence of the smooth muscle fibers of the muscularis mucosae.

2. Inside of this sheath there are always nerves, non-medullated, always large and often clustered together to form neuromata, included in connective tissue which is sometimes fibrous, sometimes edematous, sometimes hollowed out by an axial lacuna.

At first, I believed that these neuromata, so frequent in obliterated appendices, were amputation neuromata similar to those of the cerebrospinal nerves and that they were caused by division of sympathetic filaments of the mucosa by an ulcerative process. About this time, Maresch made similar observations and within a few days of each other we published almost identical papers on appendicular neuromata considered as amputation neuromata.

3. At the time when my work appeared, this explanation did not satisfy me altogether. In fact, in pursuing my studies, I had ascertained that the nerves which persisted in the appendicular axis and especially in the neuromata always contained cells, the protoplasm of which was dotted with argentaffin granules. Thus the long-sought cells were found again but this time inside of the nerves and never outside of them in the interstitial connective tissue.

This observation, the constancy of which I was able to verify, seemed to me to be of fundamental importance. It no longer permitted the interpretation of the appendicular neuromata as common amputation neuromata, for these do not contain argentaffin cells. From another point of view, it might be asked if carcinoids and argentaffin-cell neuromata were not related and even if they had not a common anatomical basis.

The Neuromata: The neuromata are composed of compound and plexiform non-medullated fibers identical with those of the intestinal mucosa, but voluminous, rolled up in masses and in close apposition. The trichrome stain colors their collagen sheath blue, the neuroglia bright red. The neuroglia is formed of ramifying and

anastomosing tubes, the contents of which, consisting doubtless of neurites, stain pale pink (Figs. 21, 22, 23, 24, 25).

The neuromata vary greatly in size. Some are microscopic, some large enough to be visible to the naked eye. They may attain a diameter of from two to three millimeters without piercing the sheath formed by the vestiges of the muscularis mucosae. Only twice have I seen neuromata which had passed this usual boundary and broken through not only the muscularis mucosae but the external muscle coat as well.

The neuromata are usually numerous; the smaller the tumors, the more easily counted. I have counted from 50 to 60 in one centimeter of appendix! When large, they tend to fuse in masses or the nerve fibers interlace in an inextricable tangle. It is important to note that they are always connected with one another by plexiform fibers, some transverse and oblique (Fig. 23), linking neuromata which have formed at the same level, others longitudinal (Figs. 23, 25), connecting tumors at different levels. In some well-oriented, longitudinal sections the appearance of these neuromata linked together recalls the nerve chain of an arthropod or worm.

Besides these interconnections the neuromata anastomose laterally and externally with Meissner's plexus of the submucosa which, however, exhibits no increase of ganglion cells or fibers.

The appearance of the neuromata varies in different specimens. Some are formed of slender fibers, rich in Remak's nuclei; these are evidently in active growth. Others consist only of large fibers with scanty nuclei; these seem to have completed their growth (Fig. 22). Others again are invaded by lymphocytes; the fibers are widely separated, shrunken and clearly in full retrogression (Fig. 21).

It is probable that the life of these neuromata is ephemeral and that they disappear one after another more or less completely; but this solitary retrogression and lymphoid infiltration would occur only if each of them had a certain autonomy in spite of its connections with its companions and with Meissner's plexus. Almost every obliterated appendix examined contained at least vestiges in the form of longitudinal and axial nerve fibers.

Are these structures really nerves and neuromata? If the answer is sought in Cajal's or Bielschowsky's technique of silver impregnation and reduction, these are not neuromata; for silver never colors neurites in them. All my attempts in this direction have failed,

as well as those of Schweizer and other workers. Schweizer concluded that these supposed neuromata are nothing but neurinomata (Schwannomata); others who, disregarding my warning, have used only the silver-reduction methods in studying obliterated appendices, have seen no nerves whatever and have simply denied my statements (Lauche).

Silver impregnation is the most capricious of all histological methods. On occasion, it colors neurites, neuroglia, elastic fibers, reticulum, collagen, we know not how or why. The many modifications of silver technique suffice to show its unreliability. As for coloring neurites, outside of the central nervous system the results are so inconstant and imperfect that I refuse to accept them as characteristic. When silver reveals black fibers in the nerves, I believe indeed that they are neurites; if silver shows nothing in a tissue which other methods, the trichrome stain especially, stamp as nervous, I reject the negative evidence. Moreover, as we shall see later, if the mucous membrane of the normal appendix is treated by Cajal's or by Bielschowsky's method, very few neurites take the stain, although the trichrome stain reveals an abundance of non-medullated fibers. It must be conceded then, not that there are no nerves in the intestinal mucosa, but that the greater number of nerves in the intestinal mucosa escape silver impregnation; now, by their topography, as we shall see, the neuromata of the obliterated appendix belong to this plexus of the intestinal mucosa.

There is another argument against the neurinoma hypothesis. All neurinomata with which we are familiar arise from localized proliferation of the Schwann cells; they contain no argentaffin cells; they are independent of one another; they do not regress. The nerve tumors under consideration communicate by longitudinal fibers, uniting one with another, and they regress individually as if, in spite of their continuity, they enjoy a certain autonomy. Hence the hypothesis that they are true neuromata, just as the nerves of the mucosa are true nerves notwithstanding the absence of argentophil neurites; and that their trophic center is neither Meissner's nor Auerbach's plexus, neither of which participates in their growth or in their degeneration.

Argentaffin cells: Argentaffin cells are always present in the growing or fully developed axial neuromata of the obliterated appendix, either singly or in groups of from two to twenty cells of the most

varied forms. Some are polygonal from reciprocal pressure, or more or less rounded.

They are enclosed in a neuroglial syncytium but their external limits cannot be determined exactly. They seem to form a part of the syncytium. Their nuclei are similar to those of carcinoid cells. Their cytoplasm is loaded with silver-reducing chromaffin, siderophil and acidophil granules, and often hollowed out by vacuoles containing lipoids. The droplets escape from the cell bodies like a product of secretion and disappear in the neuroglia. To this peculiar process of intranervous internal secretion I have given the name *neurocrinia*. Cells which belong to this type I call *neurocrine cells* (Fig. 11).

Other cells have no distinct cytoplasmic body; their nuclei are enclosed directly in the neuroglial syncytium and are surrounded by a few silver-reducing granules. These argentaffin cells are no longer individualized, they form an integral part of the Schwannian syncytium (Figs. 9, 10).

Other cells have the clear contour of ganglion cells, the nucleus at times vesicular, poorly chromophil and provided with rounded nucleoli. Only their silver-reducing granules identify them as argentaffin cells. Basic blues may show angular figures resembling Nissl bodies. I call them *cells of ganglion type* (Fig. 10).

Still other cylindrical or cuboid cells are arranged in a rosette around a cavity filled with a substance of colloid appearance, the whole being enclosed in the neuroglial syncytium. Only their basal region is filled with the silver-reducing granules. These cells correspond to the cells of Kulchitzky; they are *cells of the intestinal type* (Figs. 9, 10).

In short, in these neuromata we find cylindrical argentaffin cells and lipo-secreting cells as in carcinoids; but besides these we see two other types, which belong to the same strain however, argentaffin cells incorporated in the Schwannian neuroglia and argentaffin cells of ganglionic aspect. All these cells are in intimate contact with the neuroglial cytoplasm and completely separated from the interstitial connective tissue.

There is still more. If we study serial sections of neuromata in regression infiltrated with lymphocytes we never find argentaffin cells of any kind; but they are always to be found in the other neuromata. This observation together with the obvious indifference of

the ganglion cells and nerves of Meissner's and Auerbach's plexuses to the growth and degeneration of the axial neuromata of the appendix, leads me to believe that these neuromata depend exclusively on the multiform argentaffin cells contained in their fibers.

Without my former studies on carcinoids and the cells of Kulchitzky, I should have believed these neuromata to be ganglioneuromata of the sympathetic. I should have accepted the polygonal cells as paraganglionic and the cells of the ganglion type as sympathetic. As for the rosettes, I should have held them to be neuro-epithelial. Unfortunately, this latter interpretation could not have been advanced without embarassment; for the embryonic sympathetic contains no hollow rosettes with colloid contents, bordered by cells with a striated cuticle but rather groups of sympathogonia without central cavities. The rosettes, the silver-reducing granules and the colloido-secretory cells give to the neuromatous complex an especial character which is not seen in cerebrospinal ganglioneuromata or in sympathetic ganglioneuromata. On the contrary, they are too like the normal cells of Kulchitzky and the carcinoid cells to neglect to inquire into their possible intestinal origin.

(*B*) NEUROMATOSIS OF THE PERMEABLE APPENDIX: *Origin of the neuromata of obliterating appendicitis:* It was at this point that I took up the study of appendices still provided with a mucosa. It was obvious that if argentaffin cells occur in the neuromata, they had migrated into the nerves while the epithelium of which they normally form a part still existed. I began by examining healthy appendices or those supposed to be healthy, without pathological history, excised during laparotomies performed for various reasons, and then realized the peculiar richness of the periglandular nerve plexus of the appendix, as shown by the trichrome stain. This richness is maximum between the ages of 18 and 35 years and is far greater than in any other part of the intestine. The nerves of this plexus ramify in direct contact with the gland tubules deep in the reticulated tissue of the mucosa.

I made this observation also, that ammoniacal silver nitrate nearly always reveals an occasional argentaffin cell in these nerves. Their detection may require prolonged search for there may be only one cell in thirty or forty serial sections. As already stated, I applied the methods of Cajal and of Bielschowsky to these nerves without success.

Turning to appendices with pathological history but extirpated in the absence of any acute inflammatory crisis, I found almost always an abnormal number of silver-reducing cells in the nerves of the periglandular plexus (Fig. 13), and exceptionally a few in the inner layer of Meissner's plexus. These cells may be very few and found only after diligent search, there being only one or two in four or five sections of 5 microns; in other specimens, they are innumerable, either scattered or massed together, always inside of the nerve filaments and never in the interstices of the lymphoid tissue (Fig. 2).

In the periglandular nerves, these cells have the same characteristics as those already described in the neuromata; they may be neurocrine, Schwannian, ganglionic or cylindrical. These latter are not constant, the neurocrine cells always predominating.

Finally, the nerves inhabited by the cells are broadened, hypertrophied and increased in number. In some specimens they form actual neuromata, diffuse or localized, which push the muscularis mucosae before them (Figs. 16, 17). Their fibers are closely approximated, narrowing the meshes of the reticulum. Such a neuromatous mucosa has a characteristic clearness owing to a diminution in the number of lymphoid cells.

The neuromata are often multiple. They are situated solely beneath the gland tubules, never in the lymphoid follicles. They are connected with Meissner's plexus by non-hypertrophied fibers which traverse the muscularis mucosae. In these neuromata a certain number of neurites may be impregnated with silver, especially about their connections with Meissner's plexus, but many of them take the silver no more readily than do the neurites of the normal gland plexus or of the neuromata of the obliterated appendix.

A step further, in studying *incompletely obliterated appendices*, I found that their neuromata were continuous with the plexus of the mucosa and that they represent an extension of the argentaffin-cell neuromata which had developed in the still permeable portion of the mucosa.

Thus the neuromata of obliterating appendicitis arise from the hyperplastic plexus of the mucosa, this plexus being inhabited by argentaffin cells. If the plexus contains the argentaffin cells, it will survive and undergo hyperplasia even when the epithelial lining of the appendix has disappeared.

Origin of the Intranervous Argentaffin Cells: It remained to dis-

cover the source of the intranervous argentaffin cells. The first thing that struck me was that no matter how great their abundance, I had never seen one in mitotic or amitotic division. Whence could they come if not from the epithelial lining? Minute examination showed me that it was indeed thus and that all the argentaffin cells enclosed in the nerves result from intranervous budding from the glands of Lieberkühn. The budding epithelia then separate from their gland matrix, migrate into the nerves of the mucosa and differentiate into the various types already described.

Budding: To demonstrate argentaffin cells in the nerves of the plexus of the mucosa is an easy task if the observer is willing to use my technique, ammoniacal silver nitrate and the trichrome stain. To trace their migration from the Lieberkühnian lining into the nerves is much more difficult and rarely possible. This will be readily understood on reviewing the conditions that must happen to coincide so that a process of such short duration and of such capricious orientation may occur at the precise moment of tissue fixation, in sufficient abundance to exhibit the various stages and in a direction that coincides with the plane of the sections. I should add that the process is momentary, that it occurs under circumstances beyond our control, usually during appendicular colic with slight inflammation, and in subjects from 18 to 25 years of age. Of the 1200 appendices examined, I found budding in only fifteen. To give an idea of the difficulty of this work, I may say that in those specimens in which the buds were most numerous, I counted on an average only one in seventeen serial sections of 5 microns thickness. That is to say, finding the buds is a matter of perseverance.

I trust that I may be pardoned for giving these details. The object is not to discourage those who may wish to control my observations but to warn them against the skepticism which might follow researches insufficiently pursued.

Figures 3, 4, 5, 6, 7, 8, 15, make unnecessary a long description of this process. Budding begins with an increase in the number of nuclei in a limited area of the lining of a gland of Lieberkühn. The multiplication of nuclei is always amitotic. It is strictly localized, either at the tip of the gland tubule or more often a little to one side. It is never seen in the annular zone where mitotic figures are found or above this point.

The nuclei invariably belong to the *indifferent cells* and in the

beginning preserve their irregular contours and customary flabby aspect. Their multiplication obliges them to arrange themselves in several layers, some in the middle zone of the cell, some close to the basement membrane. Granules are never seen in the cytoplasm of these cells the limits of which are so indistinct as to give to them as a whole a syncytial aspect.

The basal ends of two or three adjoining cells now elongate, uniting to form a small projection which first pushes before it and then perforates the basement membrane and at last finds itself directly in contact with a nerve filament of the periglandular plexus (Figs. 3, 4).

Several nuclei now migrate into this pseudopod which elongates and buries itself in the nerve. The epithelial bud thus formed may remain small, consisting of few cells, or on the contrary it may grow large and multicellular. In the latter instance it assumes a spherical form and remains for a time attached to the gland by a slender pedicle. It is then and then only that the appearance of the cell changes. The nucleus swells, the chromatin becomes regularly and finely reticulated. Silver-reducing granules appear abruptly in the cytoplasm. These changes are observed first in the cells which have pushed farthest into the nerve, the first to emigrate, then in the others (Figs. 5, 6, 7, 8).

Soon the pedicle of the bud shrinks, parts, and the cells find themselves cut off from the original gland tubule. In some lucky sections in which the tissue happened to be fixed shortly after the division of the pedicle and where the invaded nerve has been cut longitudinally, a group of argentaffin cells may be seen isolated in the nerve a few hundredths of a millimeter from the lining of the gland tubule. This nerve is in direct contact with the Lieberkühnian cells which remain in the tubule and these cells insinuate slender prolongations which blend with the neuroglia (Fig. 15). Intestinal cells and neuroglial syncytium are continuous. Such pictures have a further value; they show that the various views of budding represent the emigration of epithelial cells into the nerves and not the immigration of formerly intranervous cells into the gland tubule.

Migration: After their separation from the intestinal epithelium, the cells which have become argentaffin may remain in the vicinity of the parent gland. More often they continue their migration inside of the filaments of the subglandular plexus and by this path

reach a deeper level of the mucosa. Rarely do they reach the muscularis mucosae or the superficial branches of the submucous plexus, never beyond this point.

Migration is preceded by dislocation of the cells of the bud which, when liberated, seem to insinuate themselves between the nerve fibers. Their advance is checked here and there at a crotch of the plexus by the interlacing nerve fibers. The ganglion cells (Fig. 14), present a particularly insurmountable obstacle. When budding is very active and frequent, the nerves beneath the budding gland may be seen to be invaded by argentaffin cells which, having been separated for a time, now range themselves in rows or pile up in masses as if all of them had been arrested by the same obstacle. It is in these masses resulting from blocking of the cells at certain points, and not by multiplication that the cells adapt themselves to one another and form vesicles or rosettes with colloid contents (Figs. 8, 9, 10, 12).

Differentiation of the argentaffin cells: In recent cell groups we may observe differentiation into one or the other type, intestinal, neurocrine, ganglionic or neuroglial (Figs. 9, 10). This phase of differentiation of the intranervous argentaffin cells is short, apparently as short as the phase of glandular budding which preceded it. It is seen clearly only in rare instances where budding has been met in great activity. Budding terminated, each emigrant cell soon assumes its type. The submucous plexus is crowded with polymorphic argentaffin cells, all isolated from the epithelium and capable of persisting for a long time, doubtless for years. It is in this stable form that we most frequently find the intranervous argentaffin cells in chronic, non-obliterating appendicitis and in the axial neuromata of the obliterated appendix.

Beginnings of nerve hyperplasia: In budding appendices, the periglandular plexus presents great inequalities in the caliber of the nerve fibers, the most voluminous being precisely those which have been invaded by the argentaffin cells. The nerves are enlarged not only at the points occupied by the argentaffin cells (Figs. 7, 8, 9, 10, 11, 12) and especially by the cell masses, but also beyond the cell masses where they are constituted by nerve fibers only. The enlargement of the nerve is due to an increase in the number of its constituent fibers, a multiplication due doubtless to the presence of argentaffin cells. The hyperplasia may remain limited to a few nerves or it may in-

volve a whole region of the periglandular plexus; it may not only enlarge the fibers already existing but also produce new ones which interlace, thicken and end by forming the neuromata already described.

The growth of the nerve fibers is not attended by proliferation of the argentaffin cells which become more widely separated as the neuromata are more voluminous. There is, however, an abundant amitotic multiplication of the neuroglial nuclei, Remak's nuclei, which increase in number in proportion to the increase of the neuromatous mass. The growth remains strictly limited to the intramucous plexus; the submucous and the myenteric plexuses do not participate in any way, save for rare exceptions which I shall describe in another paper.

The emigration period: In my own experience, as already related, gland budding, precursor of nerve hyperplasia, does not take place at all times. All of the subjects on whom I made these observations were between 18 and 25 years of age, the period during which, normally, the periglandular plexus has its maximum growth. Furthermore, all of these appendices presented slight inflammatory lesions involving the mucosa or had given rise to symptoms of slight acute appendicitis several days before intervention. In order to produce abundant budding it appears that two conditions must coincide, a non-mutilating irritation of the mucosa and the active period of normal neurogenesis.

On the other hand, all appendices, even normal ones, may present an occasional argentaffin cell in their nerves and these cells also are of intestinal origin. Therefore, we may suppose that the large number of buddings observed between the years of 18 and 25 both during and following slight inflammatory crises, and represented in the sequel by the presence of argentaffin cells in great numbers in the nerves, represents an exaggeration, under the influence of an irritation occurring at a favorable moment, of a normal process of neurogenesis too discrete to be detected in the healthy appendix. Thus the excessive neurogenesis which prepares the way for neuromatosis seems to be determined by an ordinary and non-mutilating acute appendicitis.

IV. Origin of Carcinoids

During the study of so many appendices, chance has enabled me to observe many carcinoids. As stated in the beginning of this paper, I have examined 50 of these tumors. Many of them were given me by pathologist friends; all of these were large. In my own material I have found 15, some of which were of particular interest because of their small size. Five were invisible to the naked eye; all had developed in neuromata, either terminal neuromata such as are found in obliterated appendices, or lateral neuromata, contiguous to a mucosa still retaining its epithelium.

In all of these specimens I was able to convince myself (Fig. 27) that the carcinoid cell columns had resulted from proliferation of intranervous argentaffin cells of the neurocrine type. These cells, piled up in the nerve fibers, finally rupture their sheaths and infiltrate the intestitial tissue of the neuroma, then that of the submucosa. These neurocrine cells proliferating in the connective tissue assume the characteristic appearance of carcinoid cells. They still secrete fatty droplets but these accumulate in their cytoplasm making it spongy. This results, perhaps, from the impossibility of eliminating their secretion into the nerves, for they are now buried in connective tissue. Proliferating in such an abnormal medium as connective tissue, the neurocrine cells group themselves like ordinary endocrine cells.

Summary and Conclusions

1. The axial region of completely obliterated appendices often (86 per cent of the specimens) contains nerves and neuromata enclosed by a discontinuous sheath formed by vestiges of the muscularis mucosae. These neuromata always contain argentaffin cells of divers forms. If the argentaffin cells disappear, the neuromata regress and are absorbed individually, notwithstanding their connections with Meissner's plexus of the submucosa.

2. Study of partially obliterated appendices shows that these neuromata arise from the periglandular plexus and that they often continue the neuromatous evolution which had commenced when the mucosa still contained gland tubules.

3. This periglandular and subglandular neuromatosis always occurs in nerves inhabited by argentaffin cells.

4. The intranervous argentaffin cells spring from the epithelium that lines the bottom of the glands of Lieberkühn.

Reversing the order in which these observations were made suffices to reconstitute the probable and logical chronological order of the phenomena. Certain intestinal cells bud out and migrate into the nerves. Here they become argentaffin. They differentiate into various forms, cylindrical cells grouped in rosettes or vesicles, cells of ganglion type, of Schwannian type, neurocrine cells. The nerves containing them grow and become neuromata, or disappear if the cells themselves disappear.

Carcinoids result from the autonomous proliferation of the isolated neurocrine cells present in the neuromata. In short, by its neuromata and by the argentaffin cells which determine their growth and which are linked with their persistence, the periglandular plexus of the appendix exhibits a remarkable autonomy.

If the cells possessed no specific granules and if we were ignorant of their intestinal origin we would be led to believe that some of them were derived from the sympathetic system, ganglionic or Schwannian, others chromaffin and paraganglionic, and the carcinoids, issue of the latter, would be sympathetic paragangliomata as stated by Danisch. However, their silver-reducing granules, their lipoids and their entodermal origin upset this hypothesis and suggest two others:

Either the nerve plexus is of sympathetic origin and proliferates under the irritating or secretory influence of the cells which have emigrated from the intestinal epithelium (but how then shall we explain the diverse morphology of these cells and especially their invariable migration into the nerves,* never into the lymphoid interstices?); or the periglandular plexus consists of a mixture of fibers, some sympathetic, others belonging to another nervous system, autonomous and autochthonous, of entodermic origin. The fibers of this latter system, like the neuromata which spring from

* Van Campenhout has recently shown that phenomena similar to those that I have seen in the appendix take place in the fetal pancreas of various mammals; they consist in emigration of cells from the primitive endocrine islands into the pancreatic nerves. The emigrated cells constitute the "sympathetico-insular complexes" to which the author freely attributes an endocrine function. Without doubt, he is right; but we might inquire if there is not something more and if certain insular cells do not take part directly in the genesis of part of the pancreatic nervous system.

them, are refractory to silver impregnation and thus their precise origin escapes us.

In the normal state might not this origin be in certain cells which are mingled with the glandular and absorbent cells just as the olfactory fibers spring from certain cylindrical and nevertheless ganglionic cells of the pituitary epithelium? From this point of view we could understand their proliferation and their elective migration into the nerves which are already in continuity with them, their forms of differentiation following exclusion from the epithelium, and the growth of the nerves.

Is it forbidden to consider the possibility of a neurentoderm, an entodermic placode, of which the cells of Kulchitzky are the sole manifestation in normal conditions? In this hypothesis, the neuromata of neurogenic appendicitis on the one hand and the carcinoids (paragangliomata of the neurentoderm?) on the other hand would illustrate the complexity of this nervous system which permeates the entire intestine (the ubiquity of carcinoids proves this) but which is not demonstrable with our present technique.

My hypothesis of the autonomy of a part of the periglandular plexus receives indirect confirmation from the demonstration of a neuromatosis which is undeniably linked with the sympathetic system. These neuromatoses are very different from those described in these pages. I shall reserve their description for a future paper.

Technique

Fixation:

Bouin's Fluid

Formol	10.
Water	30.
Glacial acetic acid (or 10 per cent trichloracetic acid)	2.
Picric acid	to saturation

Optimum fixation time: 3 days.

Do not wash in water. Dehydrate immediately in alcohol and embed in paraffin.

Regaud's Fluid

Formol	20.
3 per cent aqueous solution of potassium bichromate	80.

Optimum fixation time: 24 hours.

Wash in running water. Embed in paraffin.

Sections: Sections of 5 microns should be numerous and in series. The lesions to be studied are always localized; we must multiply the chances of finding them and be able to study them in three dimensions. The buds and the neuromata cannot be understood without many serial sections.

Affixing the sections: Dissolve 0.05 gm. gelatin (in practice, a bit of ordinary sheet gelatin one-fourth inch square) in 20 cc. distilled water, warming the water over the flame. Filter a few drops of this solution on the slide and float the section on it. Place the preparation on the warm plate at 40° C. As soon as the section spreads, remove the slide, hold the section in place with a brush or needle and stand the slide upright to drain. Blot with absorbent paper and dry in the oven at 40° C in formalin vapor secured by leaving in the oven an open bottle of formalin.

This is the only method which gives perfect adhesion of the sections no matter what the fixative, the duration of the stain, or the temperature employed.

Staining:

The Trichrome Stain

First stage: Staining the nuclei with iron hematoxylin.

The sections, freed from paraffin by toluol, alcohol and water, are immersed in 5 per cent iron alum previously heated to 45 or 50° C for 5 minutes. Wash in water.

Stain for 5 minutes at 45 to 50° C in Regaud's hematoxylin.

Hematoxylin	1.
Alcohol 95 per cent	10.
Glycerin	10.
Distilled water	80.

Rinse with 95 per cent alcohol.

Differentiate in picric alcohol, which is more selective than iron alum.

Alcohol 95 per cent saturated with picric acid	2 parts
Alcohol 95 per cent	1 part

Wash in running water.

Second stage: Staining the cytoplasm and the collagen.

Prepare the following solutions:

(*A*)	Acid fuchsin	0.3
	Ponceau de xylidine*	0.7
	Distilled water	100.
	Glacial acetic acid	1.
(*B*)	Phosphomolybdic acid	1.
	Distilled water	100.
(*C*)	Glacial acetic acid	2.
	Distilled water	100.
	Aniline blue	to saturation

Stain in *A* for 5 minutes.

Rinse with distilled water.

Differentiate in *B* for 5 minutes.

Without rinsing, pour 10 drops of *C* on the section, tilt the slide a few times for thorough mixing and let stand for 5 minutes.

Rinse in distilled water.

Back to *B* for 5 minutes.

In 1 per cent acetic acid water for 5 minutes.

Dehydrate, clear and mount in salicylic balsam.

Results: Nuclei black; argentaffin granules black or red; cytoplasm and neuroglia vermilion red; collagen intense blue.

SILVERING THE REDUCING GRANULES

(*A*) *Silvering the sections:*

Fixation: Despite the advice of many writers, tissue that is to be treated with silver should not be fixed in potassium bichromate, which in my opinion gives poor results. Formol preserves the granules very well but the delicate cytoplasmic structures very badly. The picro-acetic formol of Bouin is the fixative of choice.

Prepare the ammoniacal silver nitrate solution as follows:

To 100 cc. of a 20 per cent aqueous solution of silver nitrate, add aqua ammonia drop by drop, shaking well, until the precipitate of silver oxide is just dissolved; then add a few drops of the 20 per cent nitrate until there is a persistent opalescence. The fluid should have no odor of ammonia. Add distilled water to 200 cc., the solution now containing 10 per cent of silver nitrate. Keep in a strictly clean glass bottle and filter just before use.

* Of the brand *Microcolor*, 35 rue Escudier, Boulogne-sur-Seine (Seine), France. For this purpose, other brands of ponceau are very inferior.

The sections, affixed to slides with gelatin, are immersed in toluol, alcohol and then in distilled water, then in the ammoniacal silver nitrate at room temperature in the dark.

In 4 or 5 hours, the argentaffin granules turn yellow, then brown. In 24 hours or in 36 hours at the longest, the silver is completely reduced. Within this time limit, the silver is reduced also on certain coarse granules sometimes contained in the macrophages of the reticulum, granules of lipofuscin or of purin products, all very different from the fine granules of the argentaffin cells. Too long immersion colors the nuclei and the connective tissue.

After immersion in the ammoniacal silver for 24 hours or 36 hours at the longest, the sections are washed in water and toned in the gold bath as follows:

(*A*)	Ammonium sulphocyanide	6.
	Distilled water	100.
(*B*)	Sodium hyposulphite	6.
	Distilled water	100.
(*C*)	Gold chloride	2.
	Distilled water	100.

Mix 1 cc. *A* with 1 cc. *B*; add *C* until there is coarse precipitation and pour over the sections. Toning is instantaneous.

Rinse with 6 per cent hypo. Wash in running water.

Result: The argentaffin granules are opaque black.

It is well to stain the background, either with Cajal's picro-indigo-carmine or, better, by the second stage of the trichrome as described above, ponceau-acid fuchsin-phosphomolybdic acid-aniline blue. I recommend the latter especially; for it brings out all the tissue elements perfectly.

(*B*) *Silvering in the block:*

Fix in Bouin for 3 days.

Cut slices 2 to 3 mm. thick; wash them in running water for 24 hours.

Immerse for 24 hours in a solution of 2 drops aqua ammonia in 100 cc. distilled water.

Immerse for 24 hours in the ammoniacal silver nitrate diluted with 3 volumes of distilled water.

Rinse in distilled water.

Tone in Cajal's mixture for 24 hours:

Ammonium sulphocyanide	3.
Sodium hyposulphite	3.
Distilled water	100.
Gold chloride (1% sol.)	1.

Wash in water for several hours, embed in paraffin or celloidin.

All the argentaffin granules are opaque black (see the text); the nuclei are brownish. Counterstaining is unnecessary. It is scarcely necessary to add that the nerves are never impregnated by this method.

SUPPLEMENTARY METHOD

Impregnation of the neurites in appendices fixed in Bouin:

Fix as usual in Bouin for 3 days.

Preserve the tissue in 5 per cent neutral formol for 2 months.

At the end of this time, cut slices from 1 to 2 mm. thick, wash them in pure distilled water for 12 hours and then for another 12 hours in a mixture of 2 drops aqua ammonia in 200 cc. distilled water.

Immerse in the ammoniacal silver so diluted as to contain from 2 to 2½ per cent of silver nitrate and keep for 6 days in the dark.

Reduce for from 6 to 12 hours in:

Formol	5.
Pyrogallol	0.5
Water	50.

Embed in paraffin and section.

This method is inconstant and capricious, as are all methods with reduced silver. Its sole advantage and sole indication is that it sometimes brings out clearly the sympathetic neurites of the appendix after picro-formol fixation, which is never accomplished by the technique of Cajal or of Bielschowsky under the same conditions. It does not blacken the neurites (neuroentodermic?) of the mucosa or of the neuromata. It demonstrates the silver-reducing granules but not specifically; for the pyrogallol reduces the silver on the most diverse tissue elements, the nuclei, sometimes the peripheral neuroglia, the various pigments, etc.

This method is of secondary importance; its results should be controlled strictly by my other methods and, in any case, it should

never be trusted alone. I mention it here chiefly to correct an error of Sprafke, to whom I would recommend this method as the method of choice in the study of neurogenic appendicitis.

The photomicrographs are the work of Dr. Charles Simard, to whom I am greatly indebted for his cordial collaboration.

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DESCRIPTION OF PLATES

PLATE 44

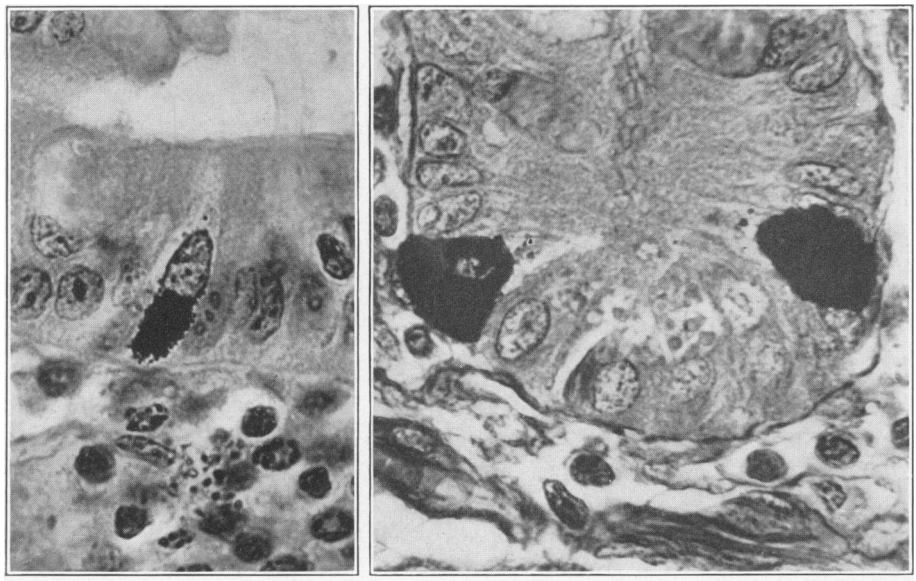
FIG. 1. Chromo-argentaffin cells of the normal intestine.

On the right, section of jejunum after silver treatment in the block. Tip of gland of Lieberkühn with two chromo-argentaffin cells, the granules relatively large and opaque, massed in the basal portion of the cell. The granules conceal the sides of the nucleus; a few are found above the nucleus. The reticulated tissue of the jejunal submucosa contains a few delicate nerve filaments, much fewer than the appendicular mucosa, even normally.

On the left, duodenum. Silvering of the sections. Argentaffin cell in the middle of a gland of Lieberkühn. Tiny uniform granules massed in the basal pole of the cell; a few above the nucleus. Note that they are much smaller in silvered sections than when silvered in the block. × 1100.

FIG. 2. Appendix of male aged 20 years, removed after three slight crises (Obs. 88c). Silvering in the block. Celloidin section.

Argentaffin cells singly and grouped in the subglandular tissue. All the cells are enclosed by nerve filaments (see next Figure). Several chromo-argentaffin cells in the gland epithelium. × 46.



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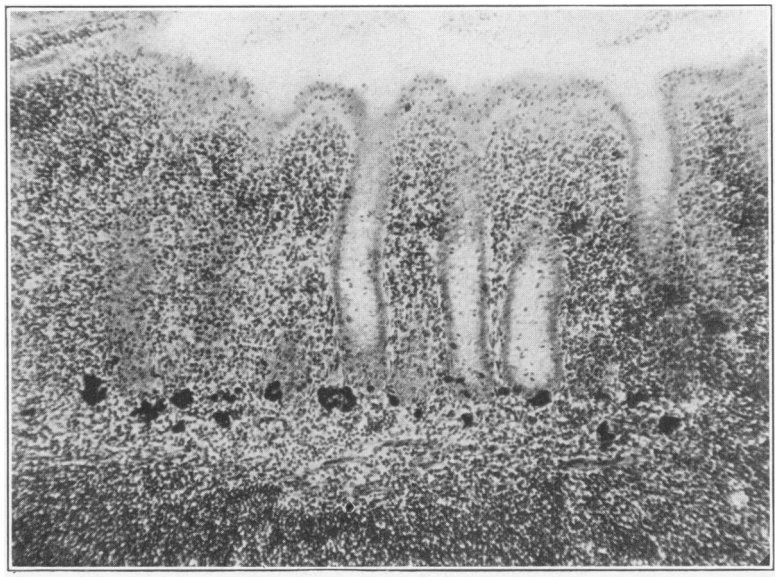

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PLATE 45

FIG. 3. Budding; initial stage (Obs. 88c). Silvering of the section; ponceau-acid fuchsin-aniline blue.

Above and to the left, the bottom of a gland of Lieberkühn. Below and in contact with it nerve filaments of the periglandular plexus. The filaments cut in various planes are surrounded by a collagen sheath, black in the photograph but blue in the preparation. Within this sheath is seen the neuroglia, gray in the Figure, red in the preparation. In cross-sections of the nerve fibers the neuroglia presents a uniformly alveolar appearance. In longitudinal section the alveoli are long, intercommunicating and tubular, running in the direction of the length of the nerve. Inside of these tubes is the pale pink, homogeneous protoplasm of the neurites. At this point there is no hypertrophy of the plexus. Note how much richer it is than in the rest of the intestine (*cf.* Fig. 1). Between the nerves are lacunae of lymphoid tissue. The gland of Lieberkühn is sprouting a bud, not very prominent as yet, formed by a syncytium with four nuclei. The cytoplasm is homogeneous and without granules. × 1100.

FIG. 4. Budding; initial stage (Obs. 88c). Silvering of the sections; ponceau-acid fuchsin-aniline blue.

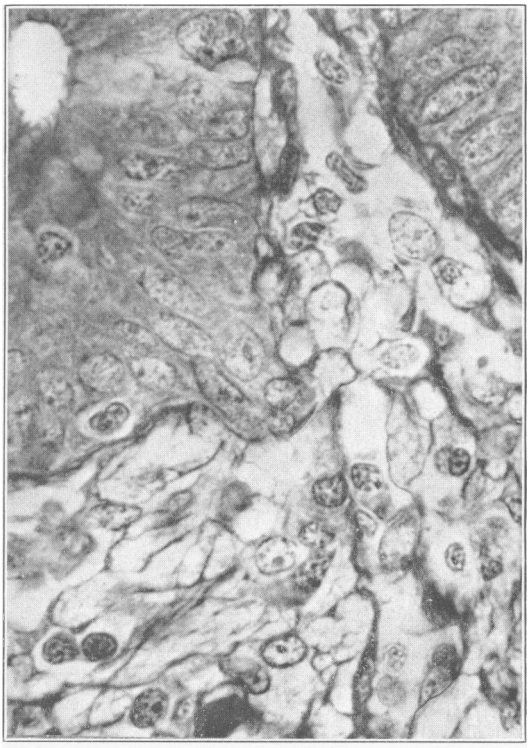
Here the bud projects directly into a large nerve; the collagen sheath of the nerve is directly continuous with the basement membrane of the gland. There are still no granules in the cells. × 1100.

FIG. 5. Budding more advanced (Obs. 88c). Silvering of the sections; magenta-picrocarmine.

Three nuclei of the bud are surrounded by an undivided cytoplasm in continuity with the lining of the gland. Only the cell which has advanced farthest into the nerve is individualized and granular. Nerve plexus hyperplastic; filaments numerous and broad. Above and to the left, an isolated nerve containing a single argentaffin cell. × 1100.

FIG. 6. Budding more advanced (Obs. 88c).

Multicellular bud in a much enlarged nerve. The four cells which migrated earliest into the nerve are individualized and granular; the others, still attached to the gland epithelium, form a multinucleated syncytial mass. × 1100.



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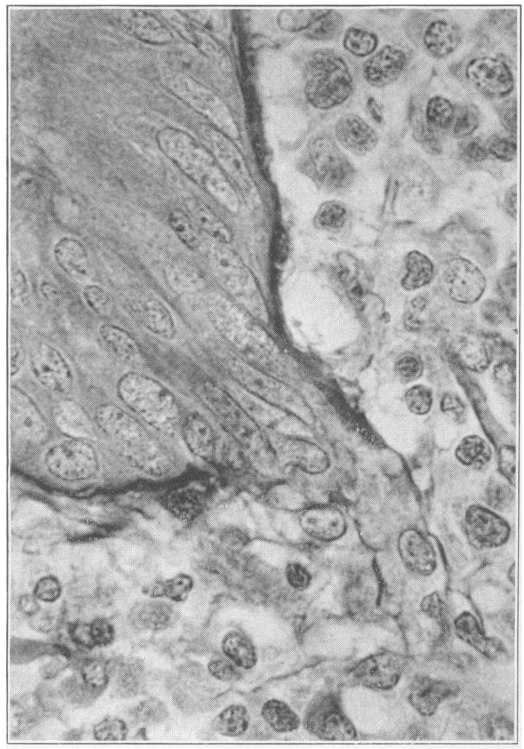

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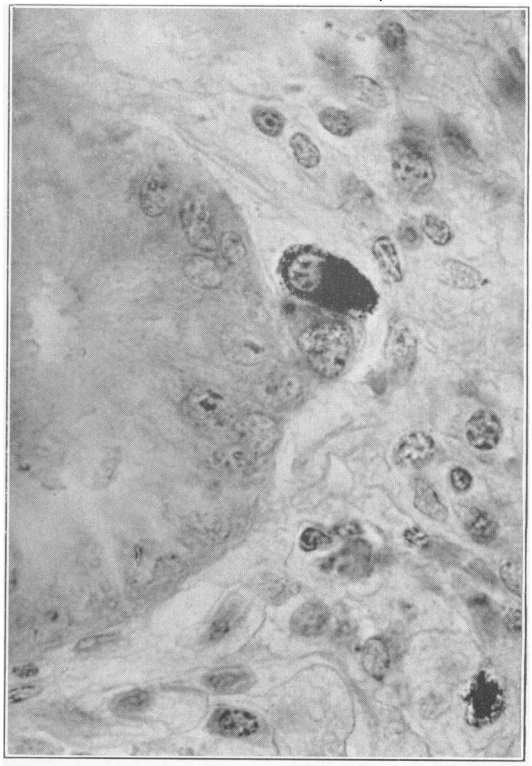

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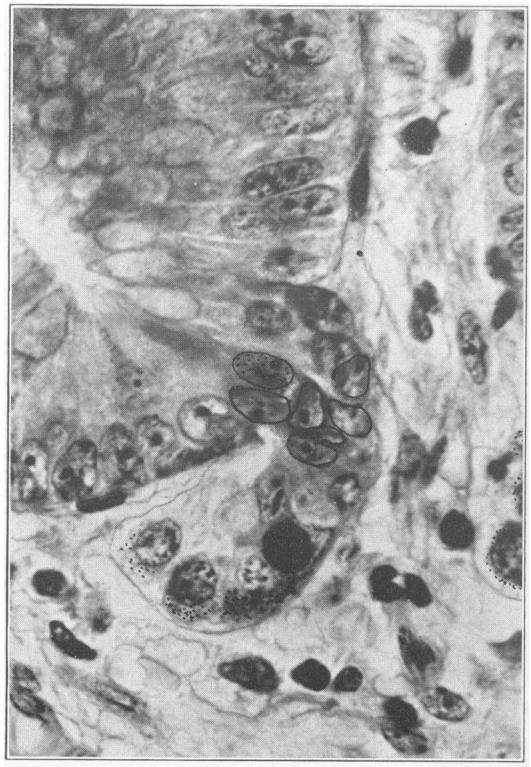

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Masson

Carcinoids (Argentaffin-Cell Tumors)

Plate 46

Fig. 7. Budding more advanced (Obs. 88c). Trichrome.

Swollen bud in a very hyperplastic nerve plexus. The bud is attached to the gland epithelium by a narrow pedicle. Only the two cells farthest advanced into the nerve are individualized and granular. × 1100.

Fig. 8. (Obs. 88c). Trichrome.

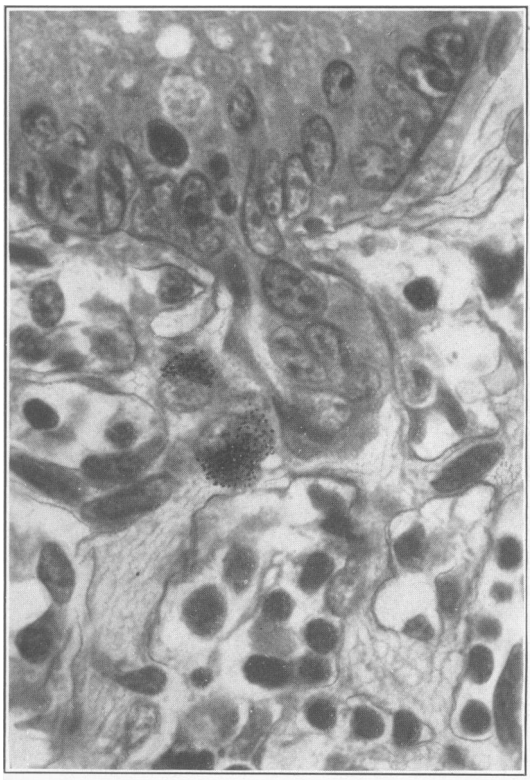
On the right, above and below, tips of glands of Lieberkühn. The left of the Figure represents the subglandular region of the reticulated tissue of the mucosa. At one side of the upper gland is a swollen nerve containing two argentaffin cells. From this same gland hangs a bud with slender pedicle ready to part. The bud is enclosed completely in a nerve bounded by its sheath. At several points the cells of the bud are separated from this sheath by neuroglia of spongy appearance. All cells are granular except those of the pedicle. Lower, argentaffin cells detached from their gland matrix and grouped around a cavity full of colloid (vesicle). These cells are enclosed in a hypertrophied nerve, their cytoplasm is continuous without clear demarcation from the neuroglial cytoplasm. × 1100.

Fig. 9. End of budding; differentiation of the argentaffin cells (Obs. 88c). Silvering of the section; ponceau-acid fuchsin-aniline blue.

Swollen bud in the gland plexus. The pedicle is formed by a large hydropic cell with an elongated nucleus staining homogeneously. Seven argentaffin cells are grouped around a cavity full of colloid. Around them and especially below them are seen elongated cells, their protoplasm containing a few argentaffin granules; they are separated from the rosette and seem to be incorporated in the neuroglia. × 1100.

Fig. 10. Vesicular group detached from the gland epithelium. Differentiation of the cells (Obs. 88c). Trichrome. Colored photograph.

In the center, a vesicle with a small cavity filled with colloid and bordered by tall cells, two of which reproduce exactly the form of the chromoargentaffin cells of the intestinal epithelium. On the right a triangular chromaffin cell having no connection with the cavity of the vesicle. Above the spherical nucleus an angular figure (Nissl body?). Below and on the left cells difficult to identify of ganglionic or neurocrine appearance. Below transition forms between argentaffin and neuroglial cells, elongation of the nuclei, effacing of cellular contours, disappearance of the granules, appearance of intracytoplasmic tubular cavities. × 1800.



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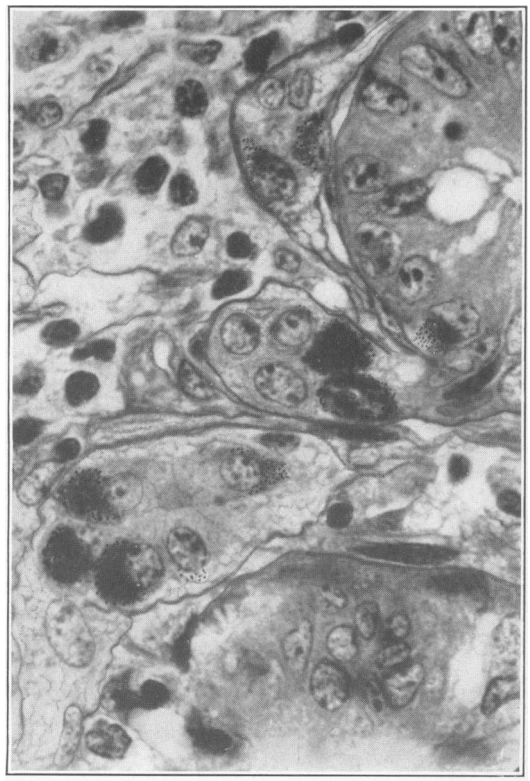

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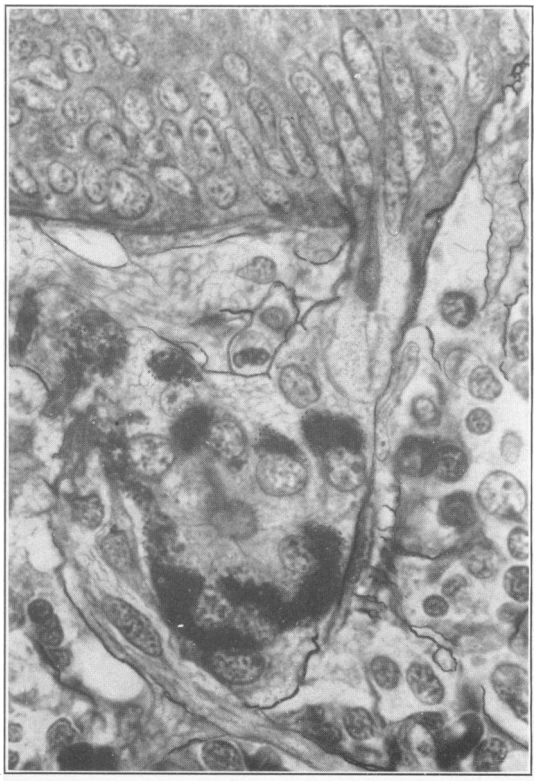

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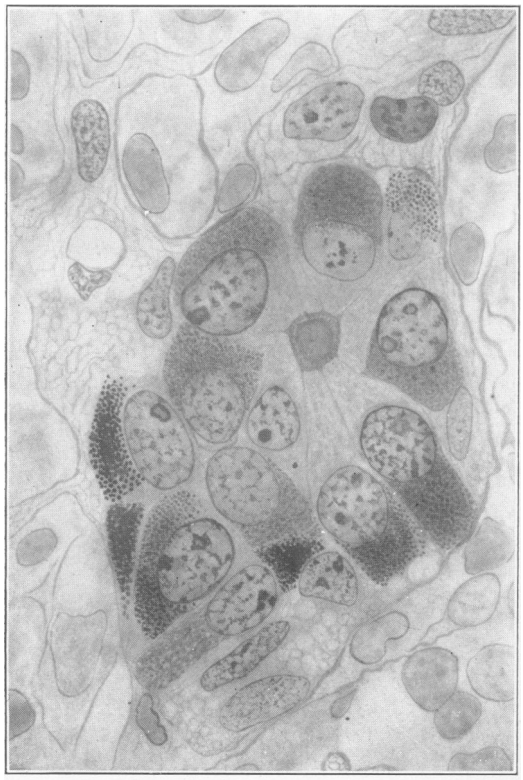

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Carcinoids (Argentaffin-Cell Tumors)

PLATE 47

FIG. 11. Neurocrinia (Obs. 167e). Formol-picric fixation; Marchi's fluid, 8 days. Magenta-picro-indigo-carmine.

Group of neurocrine cells in a hypertrophied nerve. Cells finely granular, contours indistinct, the cytoplasm continuous with that of the neuroglia. In the cytoplasm appear droplets of fat (blacken with osmic acid) which grow larger and escape from the argentaffin cells, being eliminated into the nerve where they soon disappear. They are found only in the immediate vicinity of the argentaffin cells. × 1800.

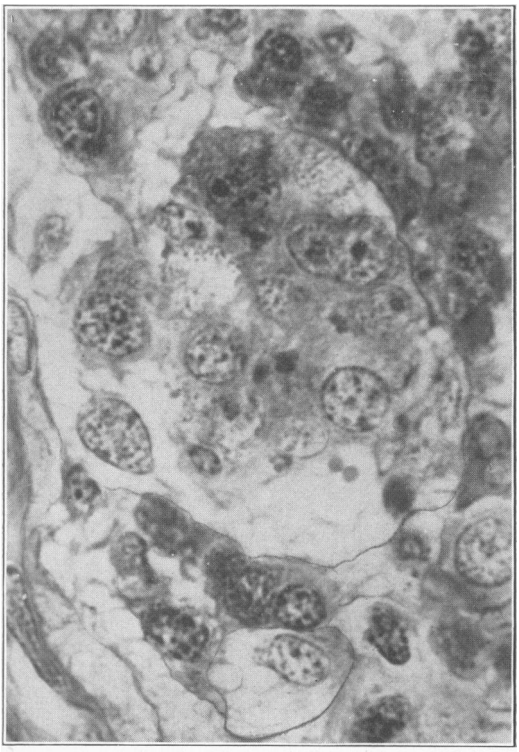
FIG. 12. Vesicle of argentaffin cells isolated in a greatly hypertrophied nerve filament (Obs. 568 ND). × 1100.

FIG. 13. Two argentaffin cells (neurocrine or ganglionic) enclosed in a nerve (Obs. 81c). Impregnation with reduced silver.

On both sides of the cells are seen four neurites which have taken the silver; it is impossible to see their relations with the cells on account of the black granules which fill the cells.

FIG. 14. Ganglion of the intramucous plexus invaded by argentaffin cells (Obs. 88c). Trichrome.

Below and on the right, two non-granular ganglion cells; on the left, two neuroglial nuclei. Above, vesicle of argentaffin cells. In the nerves which leave the upper border of the ganglion, scattered argentaffin cells. In the left upper corner, the edge of a gland of Lieberkühn from which these argentaffin cells have escaped. × 1100.



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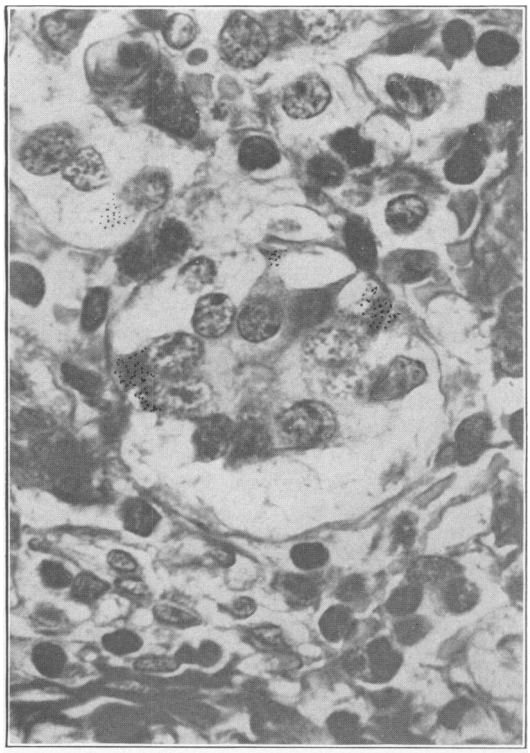

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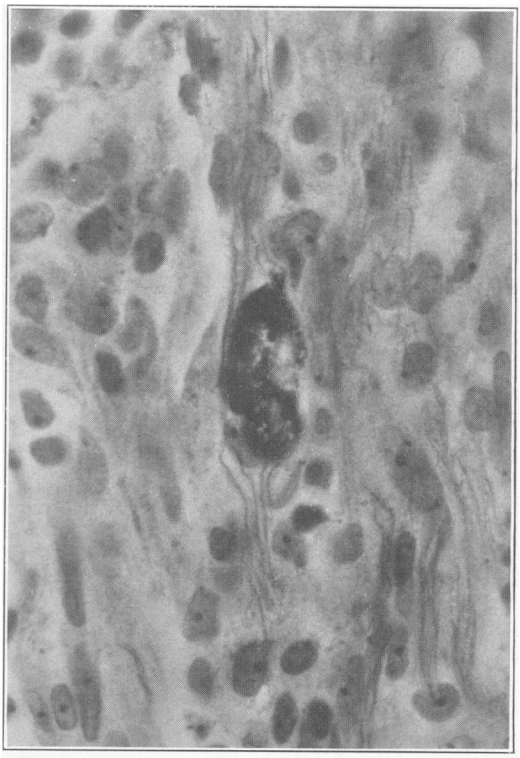

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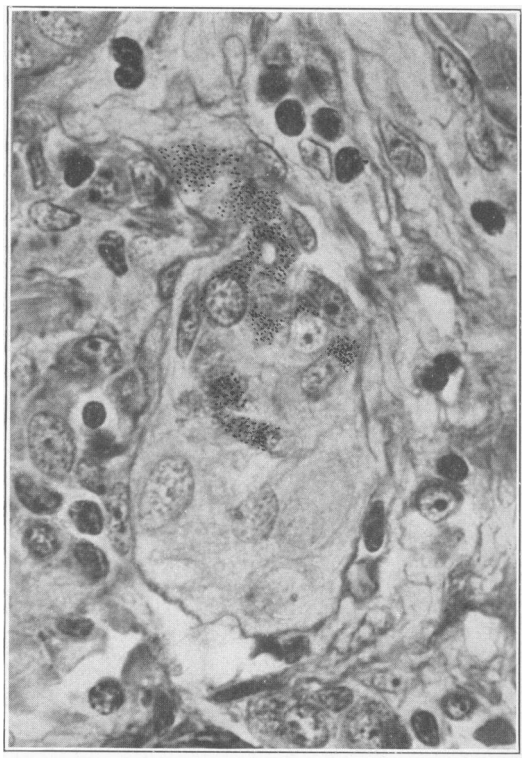

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Carcinoids (Argentaffin-Cell Tumors)

PLATE 48

FIG. 15. Connections of certain intestinal cells with the nerves persisting after budding (Obs. 88c). Silvering of the sections. Magenta-picro-indigo-carmine.

On the right and above, tip of a gland (lumen of the appendix on the right). From this tip (on the left of the Figure) hangs an intranervous bud in full activity. Below and on the right, three cells of the gland epithelium are continuous with the neuroglia of the plexus by means of slender prolongations. Near the prolongations, a round argentaffin cell. It is probable that this cell was recently detached from the epithelium at the very point where the cylindrical cells are in continuity with the neuroglia.

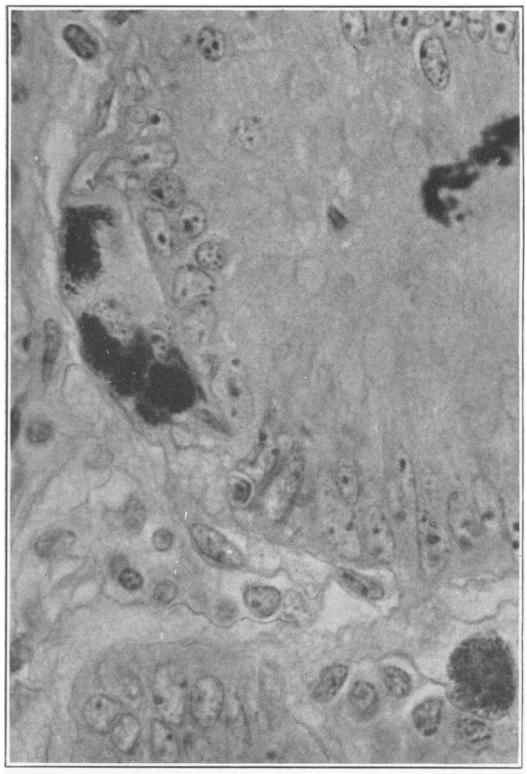
Such views show the actual and direct continuity of certain epithelial cells with the plexus. They show besides that the buds grow from the gland toward the nerve and that the process cannot be an immigration of hitherto intranervous cells into the gland (Danisch). × 1100.

FIG. 16. Neuroma formed in the depth of the mucosa, pushing before it the muscularis mucosae and the submucosa (Obs. 81c). Trichrome. Light area, the neuroma. × 48.

FIG. 17. The deep region of this neuroma.

On the left, in black, the muscularis mucosae. On the right, the neuroma. Above, an argentaffin cell of ganglion type in a node of the neuromatous plexus. × 400.

FIG. 18. Another neuroma from the same specimen, 81c. Impregnation with reduced silver. × 400.



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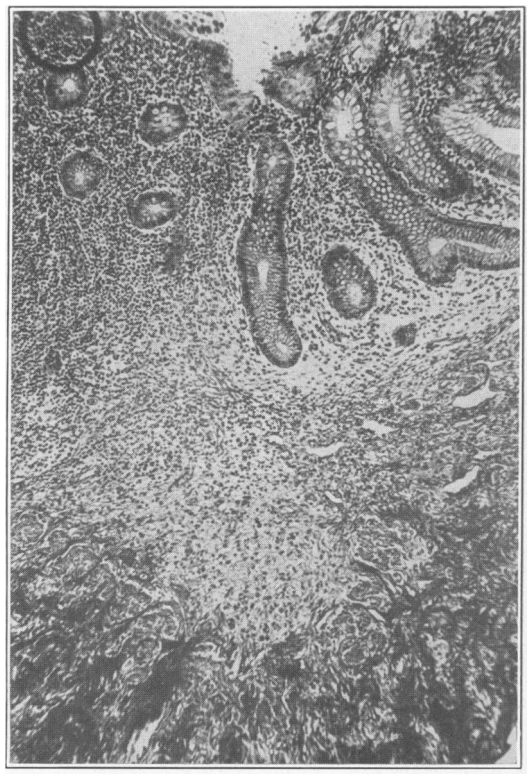

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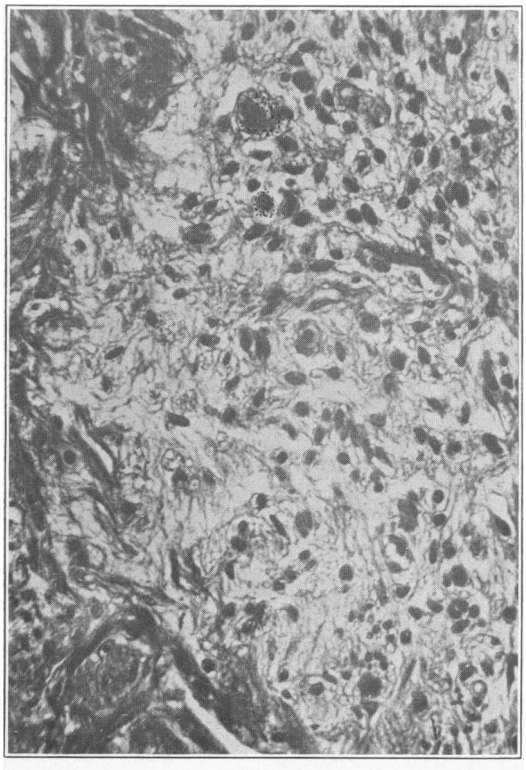

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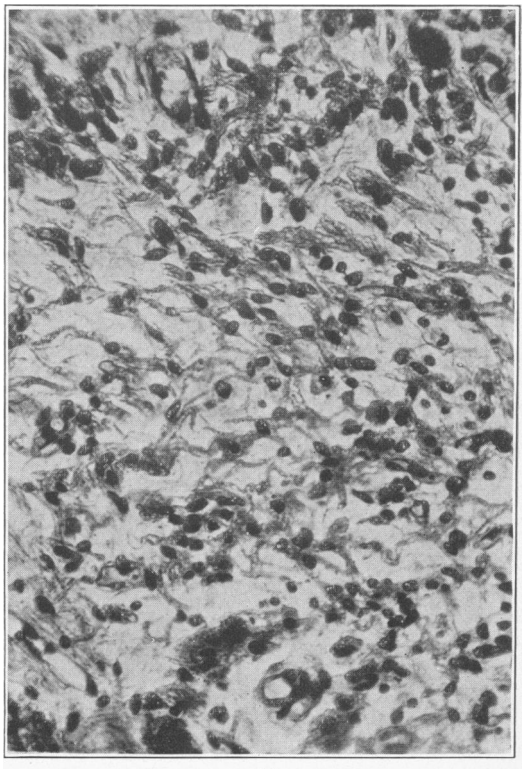

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Carcinoids (Argentaffin-Cell Tumors)

PLATE 49

FIG. 19. Appendix partially stenosed. Laterotermínal neuroma in process of growth (Obs. 228f). Trichrome.

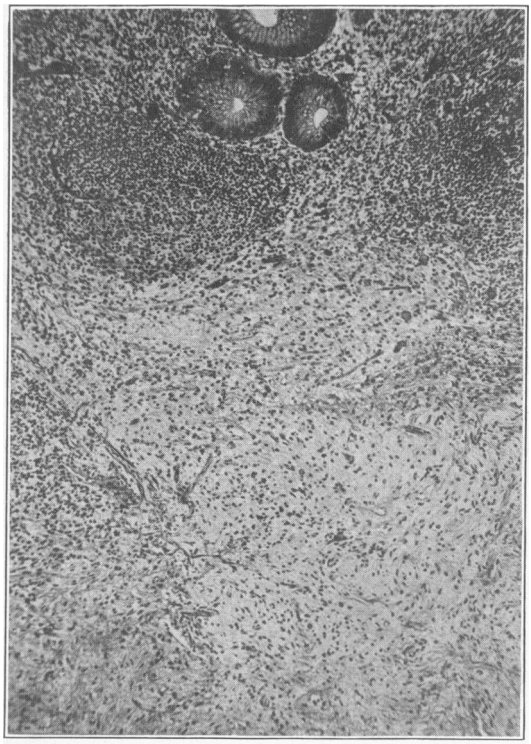
Throughout its permeable portion, the appendix contains tiny intramucous neuromata similar to those of Figs. 16, 17 and 18. At the end of the permeable region, one of these neuromata has grown quite large (light triangle occupying two-thirds of the Figure). This neuroma contains many argentaffin cells. × 48.

FIG. 20. An area in the same neuroma. × 800.

FIG. 21. Appendix completely stenosed (see Fig. 23). (Obs. 773c.) Large axial neuroma. Below and on the right, lymphoid mass representing a neuroma in regression (see Fig. 26). Trichrome. × 48.

FIG. 22. Central region of the same neuroma.

Non-medullated fibers cut across and lengthwise, anastomosing in a compact plexus. × 800.



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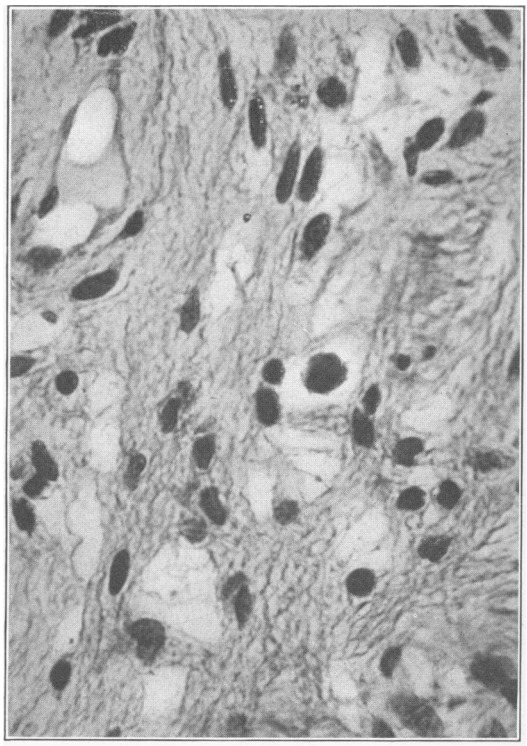

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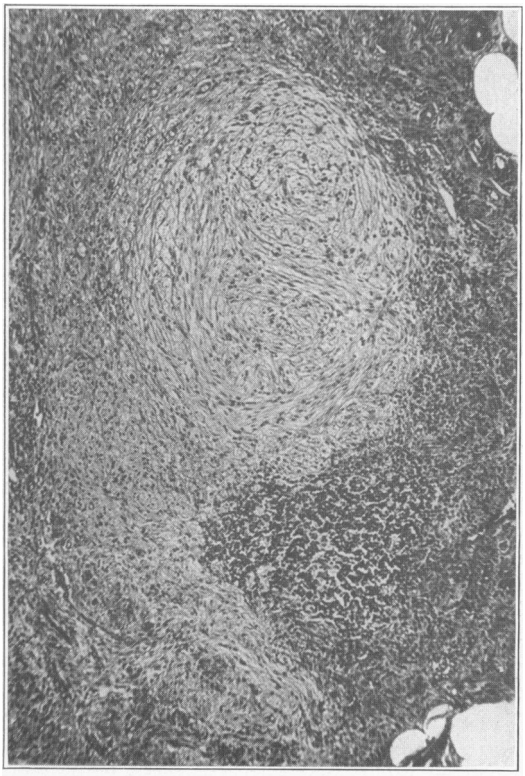

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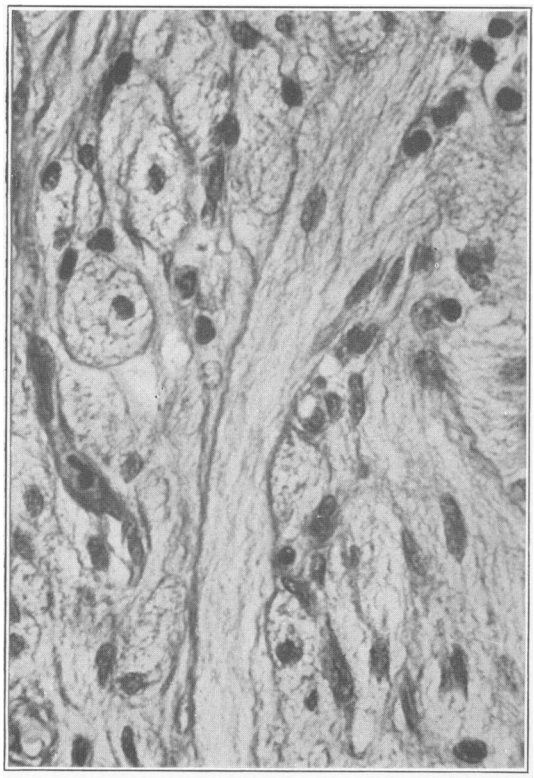

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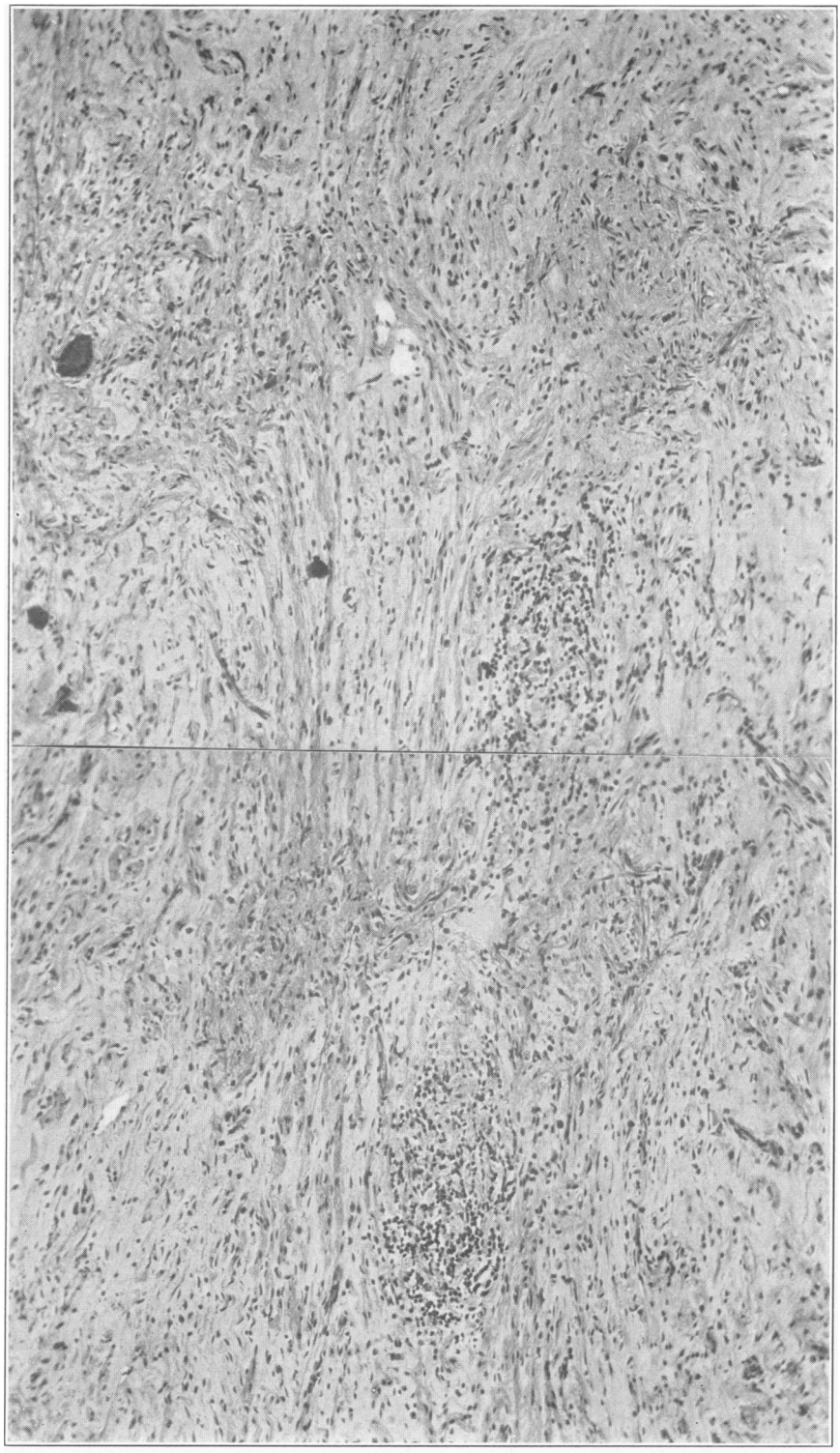
Carcinoids (Argentaffin-Cell Tumors)

Plate 50

Fig. 23. Appendix completely stenosed, distal end. Chain of neuromata formed in the axis (Obs. 773c). Trichrome.

Nerve nodules linked transversely and longitudinally by innumerable nerve filaments. In the center of the upper left neuroma, an argentaffin vesicle. The two lymphoid masses represent neuromata without argentaffin cells on the road to resorption. × 100.



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Carcinoids (Argentaffin-Cell Tumors)

PLATE 51

FIG. 24. Argentaffin vesicle (neuro-epithelial rosette?) in the neuroma of Figs. 21 and 22 (Obs. 773c). Trichrome.

The rosette is filled with colloid. Its cells are buried in the neuroglia. The black fibers represent the incomplete collagen sheaths of the nerve fibers anastomosed in an inextricable plexus. × 800.

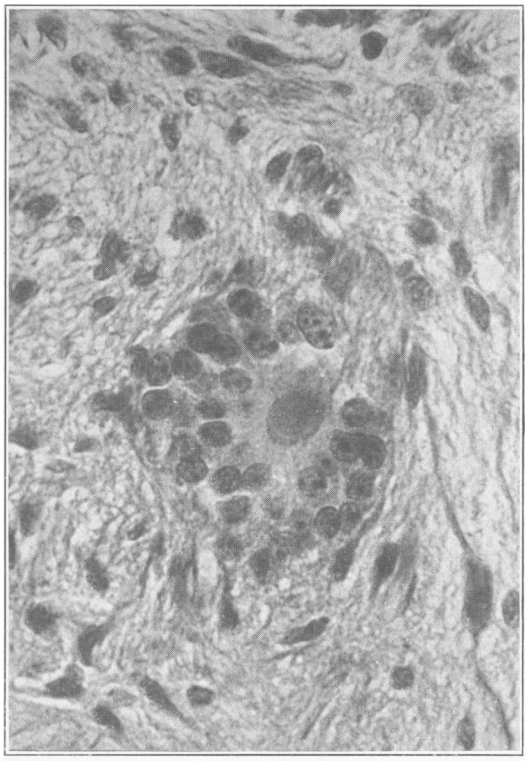
FIG. 25. Longitudinal nerve fibers linking neuroma nodules situated above and below but outside of the Figure (Obs. 69d). Impregnation with reduced silver. Here the silver has stained the neuroglia electively but not the neurites. × 400.

FIG. 26. Degenerating neuroma (Obs. 773 c). Trichrome.

Some fibers recognizable, more or less altered and separated from one another by diffuse lymphoid infiltration. × 800.

FIG. 27. Neurocarcinoid. Trichrome.

Terminal carcinoid from a child 9 years of age. Spherical tumor, 3 mm. in diameter, formed at the stenosed end of the appendix. The margin of the tumor is invading the submucosa; its center is formed by an axial neuroma. Above are seen the plexiform fibers of this neuroma. Below, these fibers are distended with neurocrine cells which are continuous with carcinoid columns, invading the connective tissue of the submucosa. × 800.



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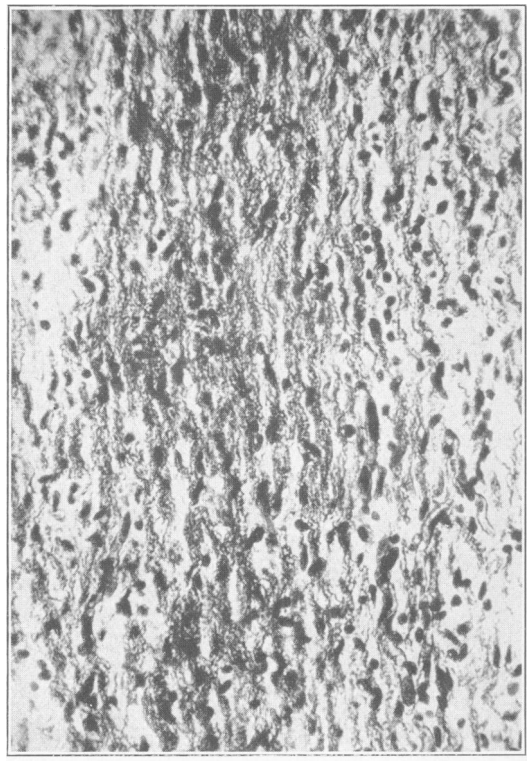

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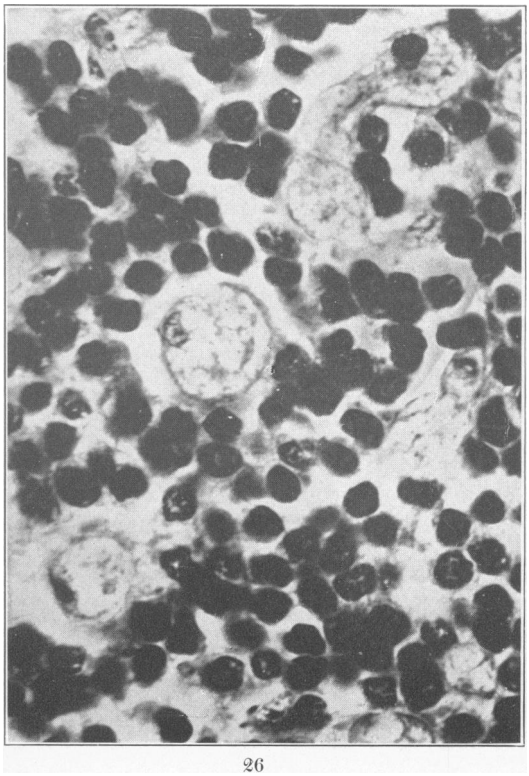

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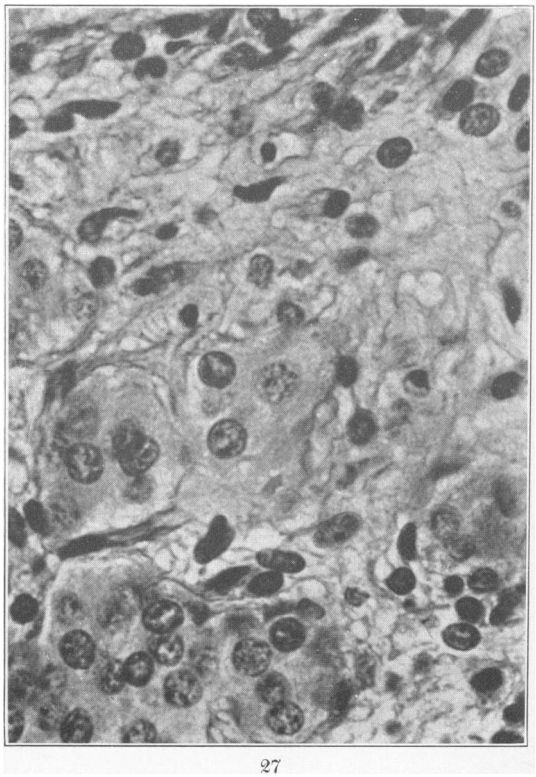

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Carcinoids (Argentaffin-Cell Tumors)

PLATE 52

FIG. 28. Carcinoid cells. Cell column with vesicles, continuous with two columns of palisade cells (Obs. 102f). Trichrome.

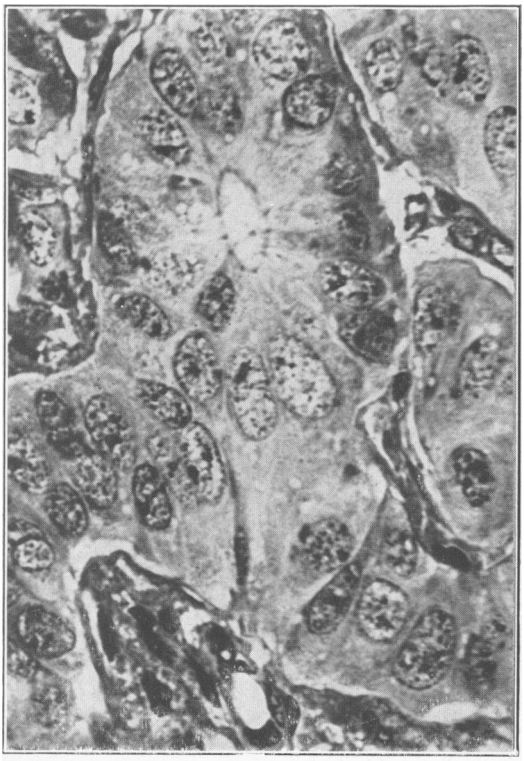
Above, vesicle formed by cylindrical cells; none of the cells figured is granular. Kittleisten are seen at the margin of the cavity. Stroma scanty and well vascularized. The long black nuclei in the stroma are nuclei of smooth muscle fibers. × 1100.

FIG. 29. Column formed exclusively by spongy cells (Obs. 102 f). Trichrome.

Resemblance to the adrenal cortex. Note the intimate connections of the spongy cell column with the capillaries (upper right corner). The protoplasm of these cells contains many argentaffin granules. × 1100.

FIG. 30. Thick column formed by spongiocytes and bordered by palisade cells. Vacuoles in their supranuclear region. In the center of the photograph, a racket-shaped palisade cell.

FIG. 31. Vesicles formed by argentaffin cells of intestinal type. Case of Delbet and Herrenschmidt. Silvering of the sections. Picro-indigo-carmine. × 1100.



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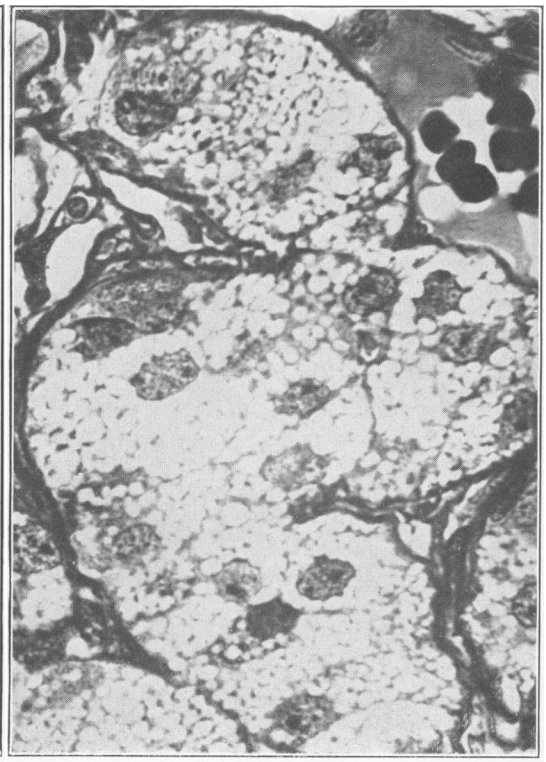

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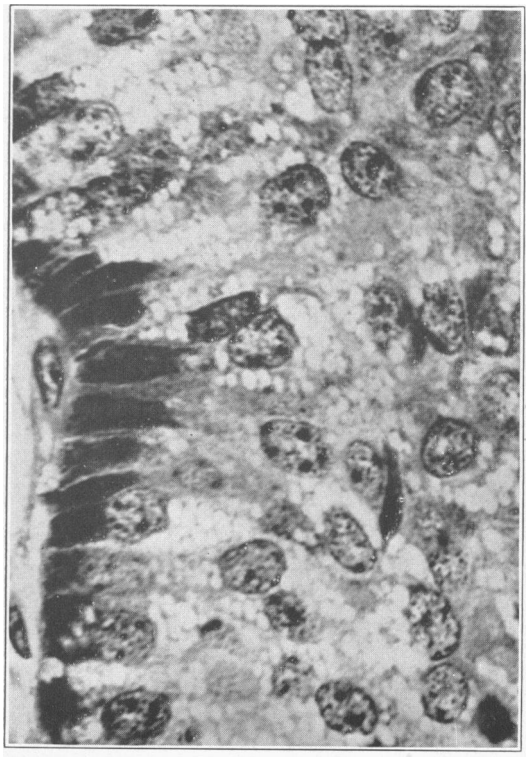

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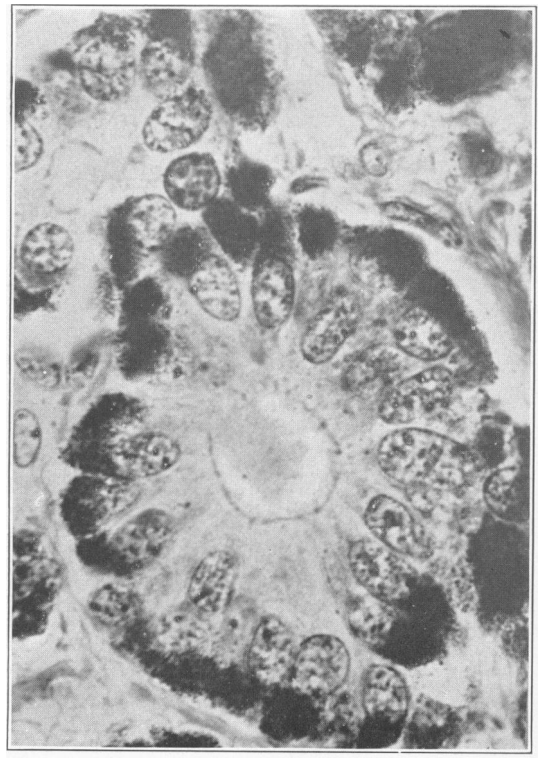

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Carcinoids (Argentaffin-Cell Tumors)

PLATE 53

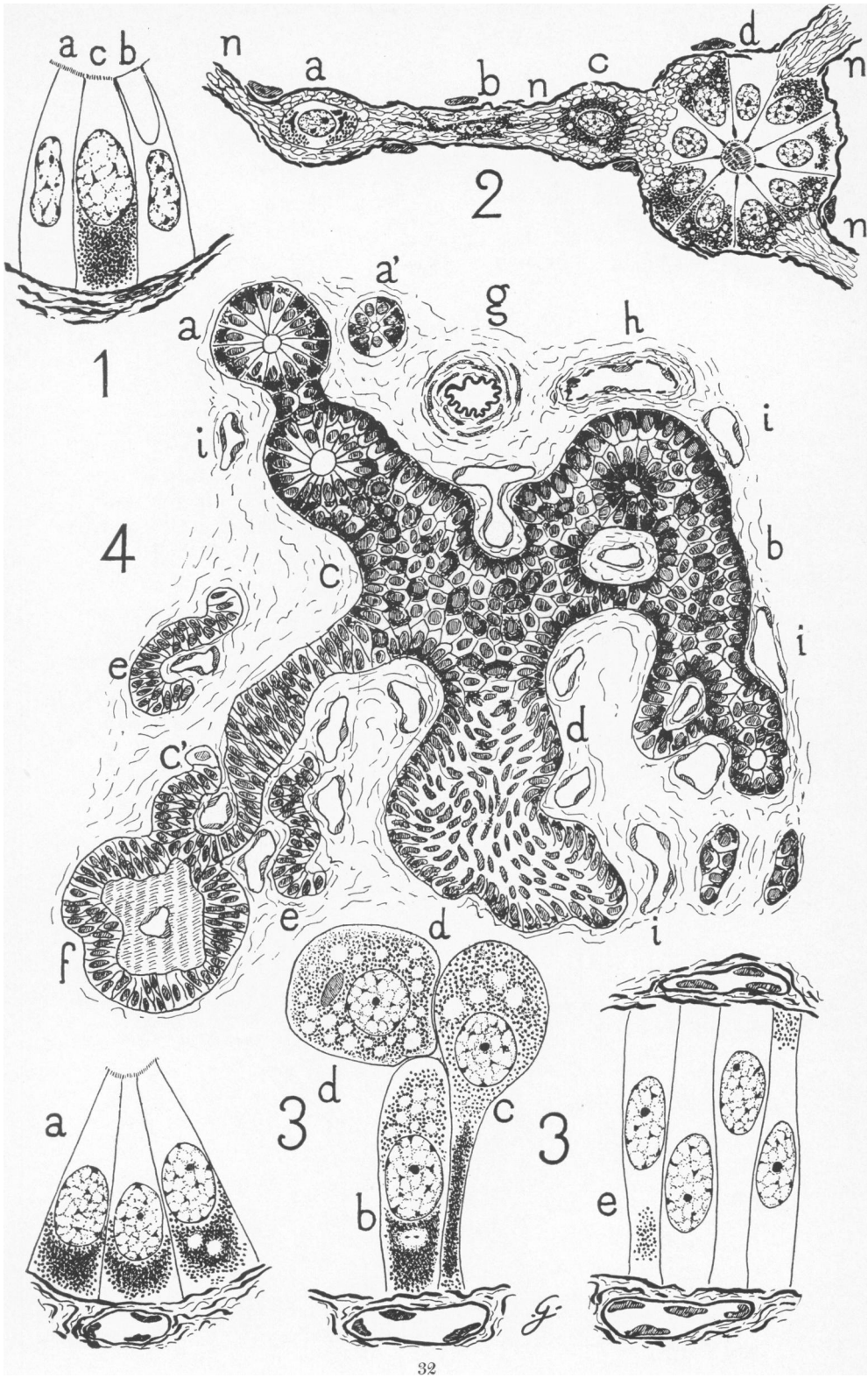
Recapitulation

FIG. 32. 1. Tip of a gland of Lieberkühn: (*a*) "indifferent" cell; (*b*) caliciform cell; (*c*) Kulchitzky cell.

2. Neuroma cells: (*n*) nerve, (*a*) cell of ganglion type; (*b*) cell of neuroglia type; (*c*) neurocrine cell; (*d*) cylindrical cells of intestinal type (Kulchitzky) forming a vesicle.

3. Carcinoid cells: (*a*) cylindrical cells of intestinal type (Kulchitzky) forming vesicles; (*b*) cylindrical palisade cell, the basal region very granular, containing a diplosome, the upper end vacuolated (lipoids) but little granular; (*c*) racket-shaped cell, swelling of the upper end, pointed at the basal end; (*d*) polygonal cell, showing central nucleus, parasome, argentaffin granules distributed diffusely and lipoid vacuoles. When numerous, these vacuoles give to the cell a spongy appearance; (*e*) cells from a pure palisade column, granules few, unevenly distributed, at only one pole of the cell.

4. Continuity of different forms of cell columns in carcinoids: (*a*) column with vesicles of cylindrical cells; (*a'*) column with vesicles of small cuboid cells; (*b*) column of polygonal cells; (*c*) palisade and polygonal cell column; (*c'*) penetration of vessels into the palisade and polygonal cell column, angiotropism; (*d*) column of palisade and fusiform cells; (*e*) pure palisade column (note relation with capillaries); (*f*) mucoid imbibition of the connective tisuse, cylindromatous evolution; (*g*) arteriole; (*h*) venule; (*i*) capillaries.



Masson Carcinoids (Argentaffin-Cell Tumors)